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(54) Title: ANTI-ANGIOGENIC GENE THERAPY VECTORS AND THEIR USE IN TREATING ANGIOGENESIS-RELATED DISEASES

(57) Abstract

A method for inhibiting tumor growth in a human patient harboring a solid tumor, said method comprising administering to said patient a nucleic acid molecule which expresses in said patient an anti-angiogenic polypeptide selected from the group consisting of human angiostatin, murine angiostatin, human endostatin, murine endostatin, and angiogenesis-inhibiting fragments thereof, wherein expression of the anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells, thereby inhibiting its growth.

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# ANTI-ANGIOGENIC GENE THERAPY VECTORS AND THEIR USE IN TREATING ANGIOGENESIS-RELATED DISEASES

## Field of the Invention

This invention relates generally to gene therapy for, e.g., cancer.

## Background of the Invention

Angiogenesis is the process by which new capillaries are formed from existing vasculature. It is a complex process which involves proliferation and migration of endothelial cells. It plays a fundamental role in reproduction, development and wound repair. Unregulated angiogenesis, however, can further the progression of many diseases, including tumor growth and metastasis, arthritis, diabetes, and some forms of blindness. For example, there is experimental evidence that limits of tumor size and growth are not the failure of the tumor cells to proliferate, but rather a failure of the tumor to provide sufficient nutrients and waste removal to its constituent cells by recruiting surrounding vasculature.

## Summary of the Invention

The invention features a method for inhibiting tumor growth in a human patient harboring a solid tumor, involving administering to the patient a nucleic acid molecule which expresses in the patient an anti-angiogenic polypeptide selected from the group consisting of human angiostatin, murine angiostatin, human endostatin, murine endostatin, and angiogenesis-inhibiting fragments thereof, wherein expression of the anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells, thereby inhibiting its growth.

In a second, related aspect, the invention features tumor inhibition, of

the type just described, using nucleic acids molecules of the formula A-B, where A and B are polypeptide and/or export signal joined by a peptide bond; peptide A contains at least 100 amino acids and includes at least kringles 1, 2, and 3 of human or murine angiostatin; and peptide B contains at least 100 amino acids and includes at least 75% of the amino acid sequence of human or murine endostatin. Expression of the fusion anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells, thereby inhibiting its growth. In some embodiments of this hybrid polypeptide and/or export signal method, polypeptide and/or export signal A further includes kringle region 4 of angiostatin, and can also include kringle region 5 of plasminogen (the larger protein molecule of which angiostatin is a portion).

In both aspects of the invention, the nucleic acid molecule preferably constitutes a portion of a viral vector or a plasmid, which can either be administered to the patient so that cells of the patient in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells are infected or transfected with the nucleic acid encoding the angiogenesis-inhibiting polypeptide, or cells (of the patient, or another human donor, or an animal) are infected or transfected *ex-vivo*, and those infected or transfected cells are then infused into the patient so that the anti-angiogenic polypeptide is expressed in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells.

As will be discussed in more detail below, in particularly effective embodiments, the nucleic acid molecule includes a nucleotide sequence

encoding a preactivation polypeptide and/or export signal for effecting Golgi and/or endoplasmic reticulum export of the anti-angiogenic polypeptide.

In another aspect, the invention features a method for treating a human patient suffering from diabetic retinopathy, involving administering to the patient one of the nucleic acid molecules described above.

The above and other features, objects and advantages of the present invention will be better understood by a reading of the following specification in conjunction with the drawings.

## Brief Description of the Drawings

Fig. 1 depicts the structural relationship of angiostatin with plasminogen.

Fig. 2 depicts the structural relationship of endostatin with collagen type XVIII.

Fig. 3 depicts various viral (A. MSCV murine retrovirus; B. Adeno-associated virus; C. HIV based retrovirus; E. recombinant adeno-virus) and non-viral (D. plasmid) vectors used in the construction of gene therapy vectors for this invention.

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| | |
|---|---|
| MSCV: | Murine Stem Cell Virus |
| LTR: | Long Terminal Repeat |
| RSV: | Rous Sarcoma Virus |
| ITR: | Inverted Terminal Repeat |
| HIV: | Human Immunodeficiency Virus |
| IRES: | Internal Ribosomal Entry Site |
| GFP: | Green Fluorescence Protein |
| HBPRE: | Hepatitis B Export Element |
| RRE: | Rev Response Element |
| polyA: | polyadenylation site |
| ψ+: | viral packaging sequence |

The inverted triangle shows the site at which the anti-angiogenic constructs will be inserted using engineered MluI and XhoI restriction sites.

* denotes specific mutations within the long terminal repeat and leader which bestows the ability for expression in embryonic stem and hematopoietic stem cells.

The arrow denotes the direction of transcription.

Fig. 4 depicts in the left (A) panel nude mice which were implanted with human neuroblastoma cells (line SK-N-AS) transduced with a mock virus and in the right (B) panel, nude mice which were transplanted with human neuroblastoma cells transduced with a retroviral gene therapy vector encoding an angiostatin-endostatin fusion protein.

Fig. 5 shows the nucleotide sequence (SEQ ID NO: 1) and amino

acid sequence (SEQ ID NO: 2) of human plasminogen and the nucleotide sequence (SEQ ID NO: 5) and amino acid sequence (SEQ ID NO: 6) of human angiostatin.

Fig. 6 shows the nucleotide sequence (SEQ ID NO: 9) and amino acid sequence (SEQ ID NO: 10) of murine endostatin.

Fig. 7 shows the nucleotide sequence (SEQ ID NO: 3) and amino acid sequence (SEQ ID NO: 4) of murine plasminogen and the nucleotide (SEQ ID NO: 7) and amino acid sequence (SEQ ID NO: 8) of murine angiostatin.

## Detailed Description

This invention provides gene therapy using a vector having a nucleotide sequence encoding one of the above-identified anti-angiogenic polypeptides. Described below in more detail are some of the components of the vectors and methods of the invention.

By a gene therapy vector is meant a vector useful for gene therapy. Gene therapy vectors carry a gene of interest that is useful for gene therapy. The gene therapy vectors are able to be transferred to the cells of an animal, e.g., a human, and are able to express the gene of interest in such cells so as to effect gene therapy. The vector can be, e.g., chromosomal, non-chromosomal, or synthetic, and can be RNA or DNA. The vector can be, e.g., a plasmid, a virus or a phage. Preferred vectors include, e.g., retroviral vectors, adenoviral vectors, adeno-associated vectors, herpes virus vectors, Simliki Forest Virus-based vector, Human Immunodeficiency virus, Simian Immunodeficiency virus, and non-viral plasmids. A preferred retroviral vector is Murine Stem Cell Virus (MSCV), which is a variant of Moloney Murine Leukemia Virus (MoMLV).

By anti-angiogenic polypeptide is meant a polypeptide which inhibits

angiogenesis. The terms polypeptide, protein and polypeptide and/or export signal are used interchangeably herein. By angiogenesis is meant the process by which new vasculature, in particular, new capillaries, are formed from existing vasculature. Angiogenesis is a complex process entailing numerous steps, including local dissolution of the basement membrane, migration of endothelial cells into the surrounding stroma, proliferation of the endothelial cells at the leading edge to form a migrating column of cells, branching and fusion of the newly formed vascular loops, and formation of a new basement membrane. By inhibiting angiogenesis is meant completely or partially inhibiting the formation of such new vasculature.

In certain embodiments, the anti-angiogenic polypeptide is an anti-angiogenic fragment of plasminogen (in particular, angiostatin), an anti-angiogenic fragment of collagen XVIII (endostatin) or a fusion of the two fragments.

Angiostatin is an internal fragment of plasminogen having a molecular weight of 38 or 45 kDa, depending on whether it contains kringles 1-3 or 1-4. In the invention, either can be used, or a molecule including kringles 1-3 and a portion of kringle 4 can be used. Angiostatin can be naturally produced in vivo in small amounts by tumor cells, e.g. murine Lewis lung carcinoma cells, by proteolytic cleavage of plasminogen so as to eliminate the N-terminal portion including the signal polypeptide and/or export signal and the preactivation polypeptide and/or export signal, as well as the C-terminal portion following kringle 3 or 4. Mouse and human angiostatin have been purified and sequenced. In preferred embodiments, the gene therapy vectors of this invention encode angiostatin having kringles 1, 2 and 3, or angiostatin having kringles 1, 2, 3 and 4.

In another preferred embodiment, the anti-angiogenic polypeptide is

endostatin or a biologically active analog or fragment thereof. Endostatin can be naturally produced *in vivo* in small amounts by tumor cells, e.g., murine angiosarcoma cells, by proteolytic cleavage of endogenous collagen XVIII so as to eliminate the N-terminal portion including the signal polypeptide and/or export signal and the preactivation polypeptide and/or export signal, as well as the C-terminal portion following kringle 3 or 4. See Fig.2. Mouse endostatin has been sequenced, and the human molecule (SEQ ID NOs: 17 and 18) forms a portion of collagen 18 (SEQ ID NOs: 19 and 20).

The human molecule position and sequence are apparent from an alignment of the active, Lys-terminated active region of human collagen 18 with murine endostatin, such that the C-terminal lysine residues align, bringing the active endostatin sequences into alignment.

In yet another preferred embodiment, the anti-angiogenic polypeptide is an in-frame fusion of angiostatin or a biologically active analog or fragment thereof and endostatin or a biologically active analog or fragment thereof. Preferably, the angiostatin or biologically active analog or fragment is 5' of the endostatin or biologically active analog or fragment. In certain embodiments, the angiostatin-endostatin fusion proteins exhibit synergistic anti-angiogenic properties.

By fragment is meant some portion of the naturally occurring anti-angiogenic polypeptide. Preferably, the fragment is at least 20 amino acid residues, more preferably at least 50 amino acid residues, and most preferably at least 100 amino acid residues in length. Fragments include chimeric constructs composed of at least a portion of the relevant gene and another molecule. The ability of a candidate fragment to exhibit a biological activity of the anti-angiogenic polypeptide can be assessed by methods known to those skilled in the art, e.g., by its ability to inhibit proliferation of bovine capillary

cells, or by its ability to inhibit growth of primary tumor cells, e.g., as described herein. See, e.g., Example 9. Also included are fragments containing residues that are not required for biological activity of the fragment or that result from alternative mRNA splicing or alternative protein processing events.

Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end (for a terminal fragment) or both ends (for an internal fragment) of a nucleic acid which encodes the polypeptide.

In preferred embodiments, the gene therapy vector of this invention is capable of hybridizing to the native anti-angiogenesis polypeptide-encoding regions and has at least about 80%, preferably at least about 90%, and more preferably at least about 95%, sequence identity to the native nucleotide sequences, and encodes a polypeptide which has anti-angiogenic activity; or a biologically active fragment of any of the above nucleotide sequences wherein the encoded polypeptide has anti-angiogenic activity.

The nucleotide sequences of the present invention can be in the form of RNA or DNA, and the nucleotide sequence can be double-stranded or single stranded and, if single stranded, can be the coding strand or non-coding (anti-sense) strand.

The coding sequence which encodes the anti-angiogenic polypeptide can be identical to the native coding sequences, or can be a different coding sequence which, as a result of the degeneracy of the genetic code, encodes the same anti-angiogenic polypeptide.

In certain embodiments, the gene therapy vector also has a nucleotide sequence encoding a signal polypeptide and/or export signal (SP) for effecting secretion of the anti-angiogenic polypeptide. Examples of signal polypeptide and/or export signal include plasminogen signal polypeptide and/or export

signal. Preferably, the signal polypeptide and/or export signal is 5' (i.e., upstream) of the nucleotide sequence encoding the anti-angiogenic polypeptide.

Preferably, the gene therapy vector has a nucleotide sequence encoding a preactivation polypeptide and/or export signal (PAP), which is a small polypeptide and/or export signal which effects folding and secretion of the anti-angiogenic polypeptide *in vivo*. Examples of preactivation polypeptide and/or export signal include plasminogen preactivation polypeptide and/or export signal, described herein, and PAP's of other proteins in the blood clotting cascade.

Preferably, the preactivation polypeptide and/or export signal is positioned 5' of the nucleotide sequence encoding the anti-angiogenic polypeptide. In embodiments which have a signal sequence and an anti-angiogenic polypeptide, preferably the preactivation polypeptide and/or export signal is 5' of the nucleotide sequence encoding the anti-angiogenic polypeptide, and 3' of the nucleotide sequence encoding the signal polypeptide and/or export signal.

We have discovered that results obtained using constructs containing a PAP- encoding nucleic acid sequence are far superior to results using constructs lacking a PAP-encoding sequence. Our hypothesis to explain these unexpectedly superior results with PAP is that, during the complex process by which the anti-angiogenic polypeptide is expressed and processed in living cells, the PAP polypeptide and/or export signal facilitates the export of the polypeptide from the cellular Golgi apparatus and/or the endoplasmic reticulum (ER). The corollary is that, absent PAP, a significant portion of the expressed polypeptide remains trapped in the Golgi and/or ER.

The PAP exemplified herein is derived from human plasminogen; this

PAP is currently preferred. Our discovery that the use of a PAP dramatically improves results leads us to believe that other PAP's would be useful as well, and such others are therefore contemplated for use in the invention. Thus, as used herein, "PAP" refers to a polypeptide and/or export signal which is naturally associated with a eukaryotic (preferably human) protein, the exportation of which is facilitated by its associated PAP. Examples of other human proteins whose Golgi/ER export is PAP-facilitated include other secreted proteins of the blood coagulation cascade, e.g., fibrinogen, prothrombin, Factor VIII, and Factor IX. Other secreted human proteins also are associated with potentially useful PAPs.

It is not essential that the PAP used in the invention be identical in amino acid sequences to a native PAP; it is well-known that polypeptide and/or export signal that facilitate protein secretion or export, e.g., signal polypeptide and/or export signal and PAPs, can vary from the native forms to a certain extent and still retain their function. Therefore, PAPs useful according to the invention preferably have 75% or greater amino acid sequence identity with a native PAP.

In certain embodiments, the gene therapy vector has a nucleotide sequence encoding a tag for identification of the anti-angiogenic polypeptide and/or export signal. In certain embodiments, the tag is 5' of the nucleotide sequence encoding the anti-angiogenic polypeptide; in other embodiments, the tag is 3' of the nucleotide sequence encoding the anti-angiogenic polypeptide. In embodiments in which the anti-angiogenic polypeptide is endostatin or an angiostatin-endostatin fusion, it is preferred that the tag be 5' of the nucleotide sequence encoding endostatin.

In certain embodiments the gene therapy vector includes a selectable

marker, e.g., a Neomycin phosphotransferase gene, or a humanized red-shifted green fluorescent protein.

The invention also includes a cell infected or transfected with a gene therapy vector described herein. Preferably, the cell is an animal cell, more preferably an autologous or allogeneic human cell. The gene therapy vectors described herein can be introduced into a cell, e.g., by transformation, transfection, transduction, infection, or ex vivo injection. They can be targeted to a particular cell type.

Administration of nucleic acid, e.g., a gene therapy vector, can be accomplished by any method which allows the nucleic acid to reach the target cells. These methods include, e.g., injection, deposition, implantation, suppositories, oral ingestion, inhalation, topical administration, or any other method of administration where access to the target cells by the nucleic acid is achieved. Injections can be, e.g., intravenous, intradermal, subcutaneous, intramuscular or intraperitoneal. Implantation includes inserting implantable drug delivery systems, e.g., microspheres, hydrogels, polymeric reservoirs, cholesterol matrices, polymeric systems, e.g., matrix erosion and/or diffusion systems and non-polymeric systems, e.g., compressed, fused or partially fused pellets. Suppositories include glycerin suppositories. Oral ingestion doses can be enterically coated. Inhalation includes administering the nucleic acid with an aerosol in an inhalator, either alone or attached to a carrier that can be absorbed.

In certain embodiments of the invention, administration can be designed so as to result in sequential exposures to the nucleic acid over some time period, e.g., hours, days, weeks, months or years. This can be accomplished by repeated administrations of the nucleic acid, e.g., by one of the methods described above, or alternatively, by a controlled release delivery system in

which the nucleic acid is delivered to the animal over a prolonged period without repeated administrations. By a controlled release delivery system is meant that total release of the nucleic acid does not occur immediately upon administration, but rather is delayed for some time. Release can occur in bursts or it can occur gradually and continuously. Administration of such a system can be, e.g., by long acting oral dosage forms, bolus injections, transdermal patches or subcutaneous implants. Examples of systems in which release occurs in bursts include, e.g., systems in which the nucleic acid is entrapped in liposomes which are encapsulated in a polymer matrix, the liposomes being sensitive to a specific stimulus, e.g., temperature, pH, light, magnetic field, or a degrading enzyme, and systems in which the nucleic acid agent is encapsulated by an ionically-coated microcapsule with a microcapsule core-degrading enzyme. Examples of systems in which release of the nucleic acid is gradual and continuous include, e.g., erosional systems in which the nucleic acid is contained in a form within a matrix, and diffusional systems in which the nucleic acid permeates at a controlled rate, e.g., through a polymer. Such sustained release systems can be, e.g., in the form of pellets or capsules.

The nucleic acid is administered to the patient in a therapeutically effective amount. By therapeutically effective amount is meant that amount which is capable of at least partially preventing or reversing the disease. A therapeutically effective amount can be determined on an individual basis and will be based, at least in part, on consideration of the patient's size, age, the efficacy of the particular nucleic acid used, the type of delivery system used, the time of administration relative to the onset of disease symptoms, and whether a single, multiple, or controlled release dose regimen is employed. A therapeutically effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

In certain embodiments, a therapeutically effective amount of an anti-angiogenic polypeptide is administered by providing to the animal a nucleic acid encoding the polypeptide and expressing the polypeptide in vivo. Nucleic acids encoding the polypeptide, or mutants thereof, can be administered in any biologically effective carrier, e.g. any formulation or composition capable of effectively delivering the nucleotide sequence for the anti-angiogenic polypeptide to cells in vivo. Approaches include, e.g., insertion of the nucleic acid into viral vectors. Viral vectors can be delivered to the cells, e.g., by infection or transduction using the virus. Viral vectors can also be delivered to the cells, e.g., by physical means, e.g., by electroporation, lipids, cationic lipids, liposomes, DNA gun, $Ca_3(PO_4)_2$ precipitation, or delivery of naked DNA. In certain preferred embodiments, the virus is administered by injection, e.g., intramuscular injection, in a dose range of about $10^3$ to about $10^{10}$ infectious particles per injection, more preferably in a dose range of about $10^5$ to about $10^8$ infectious particles per injection. Single or multiple doses can be administered over a given period of time, depending, e.g., upon the disease.

An alternative is insertion of the nucleic acid encoding the anti-angiogenic polypeptide into a bacterial or eukaryotic plasmid. Plasmid DNA can be delivered to cells with the help of, e.g., cationic liposomes (lipofectin™; Life Technologies, Inc., Gaithersburg, MD) or derivatized (e.g., antibody conjugated) polylysine conjugates, gramacidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or $Ca_3(PO_4)_2$ precipitation carried out in vivo, or by use of a gene gun. The above-described methods are known to those skilled in the art and can be performed without undue experimentation.

Since transfer of the nucleic acid to appropriate target cells represents the critical first step in gene therapy, choice of the particular gene delivery

system will depend on such factors as the intended target and the route of administration, e.g., locally or systemically. Targets for delivery of the nucleic acid can be, e.g., specific target cells which are diseased. For example, the target can be, e.g., the peritoneal cavity, gastro-intestinal tract, bone marrow cavity, liver, lungs, muscles, vasculature, pericardial cavity, pleural cavity, skin, sub-cutaneous or deep connective tissues, central nervous system, spinal fluid, eye, or specific sites of tumor growth. Administration can be directed to one or more cell types, and to one or more cells within a cell type, so as to be therapeutically effective, by methods known to those skilled in the art. For example, the nucleic acid can be, e.g., coupled to an antibody, to a ligand to a cell surface receptor, or to a toxin component, or can be contained in a particle which is selectively internalized into cells, e.g., liposomes, or a virus where the viral receptor binds specifically to a certain cell type, or a viral particle lacking the viral nucleic acid, or can be administered by local injection.

In certain embodiments, the nucleic acid is administered to the patient by introducing *ex vivo* the nucleic acid into cells of the patient, or into syngeneic or allogeneic or xenogeneic cells, and then administering the cells having the nucleic acid to the animal. Any cell type can be used. In certain embodiments, the cells having the introduced nucleic acid are expanded and/or selected after the nucleic acid transfer. The cells having the transferred nucleic acid are subsequently administered to the patient. Preferably, the cells are administered in a dose range of about $1 \times 10^6$ to about $1 \times 10^9$ cells/dosage/day, and most preferably at about $1 \times 10^7$ to about $1 \times 10^8$ cells/dosage/day. The cells can be administered by any method which results in delivering the transferred nucleic acid in the cells to the desired target. For example, the cells can be implanted directly into a specific tissue of the patient, or implanted after encapsulation within an artificial polymer matrix. Examples of sites of

implantation include, e.g., the peritoneal cavity, gastro-intestinal tract, bone marrow cavity, liver, lungs, muscles, vasculature, pericardial cavity, pleural cavity, skin, sub-cutaneous or deep connective tissues, central nervous system, spinal fluid, eye, or specific sites of tumor growth.

Systemic delivery can be achieved, e.g., by introducing the nucleic acid into cells which circulate in the peripheral blood of the patient, or which give rise to cells which circulate in the peripheral blood. In certain embodiments, the nucleic acid is introduced into such cells *ex vivo*, and these cells are then administered to the patient, resulting in systemic delivery within the peripheral blood. These cells can be the cells of the patient or allogeneic cells. Preferred cells in which the nucleic acid can be introduced are hematopoietic cells.

In certain embodiments, other therapy is additionally administered. For example, if the animal is being treated for a tumor, other tumor therapy, e.g., another therapeutic agent, chemotherapy, radiation or surgery, is additionally administered to the patient, either simultaneously or at different times.

Treating is meant to include, e.g., preventing, treating, reducing the symptoms of, or curing the disease. I.e. treating a tumor includes preventing growth of the tumor, causing shrinkage of the tumor, or preventing development of micro-metasteses.

Preferably, the recombinant nucleic acid is a gene therapy vector, e.g., as described herein. Preferably, the anti-angiogenic polypeptide is angiostatin, endostatin, an angiostatin-endostatin fusion protein, or biologically active analogs or fragments thereof. In certain embodiments, the angiostatin has kringles 1, 2 and 3; in other embodiments, the angiostatin has kringles 1, 2, 3 and 4, and, in some embodiments, kringle 5 of human or murine plasminogen. Angiostatin is described in O'Reilly and Folkman U.S. Patent No. 5,639,725, hereby incorporated by reference. Endostatin is described in O'Reilly and

Folkman PCT Appln. No. WO 97 15666, published May 1, 1997, hereby incorporated by reference.

In certain embodiments, the recombinant nucleic acid has been introduced *ex vivo* into cells so as to express the anti-angiogenic polypeptide in the cells, and the recombinant nucleic acid is administered to the patient by administering to the patient the cells containing the recombinant nucleic acid. In certain embodiments, the cells are derived from the patient; in other embodiments the cells are allogeneic cells relative to the cells of the patient.

Where cells are infected or transfected *ex vivo* for later infusion into the patient, the cells are preferably hematopoietic cells, but can also be mesenchymal cells, stem cells, epithelial cells (e.g., from the gut), or dendritic cells.

The gene therapy vectors of the invention can be provided in a pharmaceutical composition comprising a therapeutically effective amount of the recombinant nucleic acid together with a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include, e.g., water, saline, dextrose, glycerol, ethanol, liposomes and lipid emulsions.

The following non-limiting examples further illustrate the present invention.

## EXAMPLES

Example 1: Construction of Inserts for Gene Therapy Vectors Containing cDNA for Angiostatin, Endostatin or Angiostatin-Endostatin Fusion Proteins

The following genetic constructs are inserted into retroviral gene therapy vectors; the genetic constructs contain human or murine cDNA for angiostatin, endostatin or an angiostatin-endostatin fusion, and DNA encoding a signal

polypeptide and/or export signal (SP), a tag (FLAG), and, preferably, a preactivation polypeptide and/or export signal (PAP). The constructs are all made using standard genetic engineering techniques, and their insertion into retroviral gene therapy vectors is carried out using known methods. The constructs have the following components:

| Murine Constructs | |
|---|---|
| SP-K1-K2-K3-Flag | |
| SP-K1-K2-K3-K4-Flag | |
| SP-K1-K2-K3-K4-K5-Flag | |
| SP-PAP-K1-K2-K3-Flag | |
| SP-PAP-K1-K2-K3-K4-Flag | (SEQ ID NO: 11 and 12) |
| SP-Flag-Endo | (SEQ ID NO: 13 and 14) |
| SP-K1-K2-K3- Flag-Endo | |
| SP-K1-K2-K3-K4- Flag-Endo | (SEQ ID NO: 15 and 16) |
| SP-PAP-K1-K2-K3- Flag-Endo | |

Human Constructs

SP-K1-K2-K3
SP-K1-K2-K3-K4
SP-K1-K2-K3-K4-K5
SP-PAP-K1-K2-K3
SP-PAP-K1-K2-K3-K4
SP-PAP-K1-K2-K3-K4-K5
SP-Endo
SP-K1-K2-K3-Endo
SP-PAP-K1-K2-K3-Endo

Nucleic acid and amino acid sequences for mouse and human angiostatin and endostatin used in these constructs are shown in Figs. 5-7.

Nucleic acid and amino acid sequence of the FLAG peptide:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| amino terminus- | ASP | TYR | LYS | ASP | ASP | ASP | ASP | LYS |
| 5'- | GAC | TAC | AAG | GAC | GAC | GAT | GAC | AAG |

Human plasminogen derivative constructs

The entire coding region of the human plasminogen cDNA from the start (ATG) to the stop (TAA) codon is 2433bp in size.

This sequence encodes a signal peptide (bp 1-57), a preactivation peptide (bp 58-288), and 5 distinct structural regions known as kringles (K1-K3 from bp 289-1092; K4 from bp1093-1380; K5 from bp 1381-1740). Please note that although I have given precise bp measurements for kringles K4 and K5, it can be argued that the sequence encoding K4 is between bp1056-1440 and the sequence encoding K5 is between bp1362-1680.

A DNA fragment encoding a portion of the human plasminogen protein from bp 1 to 1377 was obtained by PCR of a widely available human liver cDNA library using synthetic DNA oligonucleotides complementary to sequences immediately preceding the signal peptide and immediatly following kringle 4. This fragment contains the sigal peptide (bp1-57), the preactivation peptide (bp 58-288), kringles 1 (bp289-549), 2 (bp 550-804), 3 (bp 805-1092) and 4 (bp 1093-1380). The synthetic oligonucleotides used for this reaction contained engineered recognition sites for the restriction enzymes EcoRI and XhoI. Following the PCR reaction the amplified fragment was cloned into the EcoRI/XhoI sites of BluescriptSK(-) (Stratgene) using standard techniques (Maniatis). Following cloning the integrity of the amplified sequence was verified by sequencing both strands using the Sanger method (Sanger). Various derivatives of the cloned fragment were subsequently constructed using BluescriptSK(-) (Stratagene) as a backbone. A full list of the derivatives are described in Table 1. Briefly, the variations are composed of constructs containing various combinations of kringles with or without the signal and/or preactivation peptide sequences. These derivatives were constructed using both standard techniques as well as PCR and the use of double stranded synthetic oligonucleotides. In all cases the integrity of the start codon, coding sequence and termination codon was verified by double stranded sequencing using the Sanger method.

Murine plasminogen derivative constructs

The coding sequence for murine plasminogen is 2439bp in size and, similar to the human plasminogen cDNA encompasses a sequence encoding signal and preactivation peptides (bp 1-57 and 58-288 respectively) in addition to 5 kringle regions; kringle 1-3 (bp 289-1092), kringle 4 (bp 1093-1380) and kringle 5 (bp 1381-1743). Again, although I have given precise bp measurements for kringles K4 and K5, it can be argued that the sequence encoding K4 is between bp1056-1440 and the sequence encoding K5 is between bp1362- 1680.

The murine plasminogen cDNA has previously been cloned and was made available to us. Derivatives of murine plasminogen were constructed using sequences derived from bp 1-1743 of the coding sequence. Various combinations of kringle regions with or without signal and preacivation peptide regions were made using BluescriptSK(-) (Stratagene, La Jolla, CA) as the vector backbone. These derivatives were constructed using standard cloning techniques (Maniatis, Molecular cloning; a laboratory manual, second edition, 1989) in combination with PCR utilizing synthetic oligonuleotides using

Angiostatin function was not altered by adding the FLAG polypeptide and/or export signal to either the N- or C-terminal ends, whereas endostatin was functional only if FLAG was added to its N-terminal end.

Example 2: Construction of Retroviral Gene Therapy Vectors

This example illustrates the construction of retroviral gene therapy vectors comprising cDNA for angiostatin, endostatin or angiostatin-endostatin fusion proteins.

The DNA inserts from Example 1 were inserted into two retroviral vectors. Both vectors were derived from the Murine Stem Cell Virus (MSCV), which is a variant of Moloney Murine Leukemia Virus (MoMLV) having several mutations allowing high, sustained expression in hematopoietic stem cells and their progeny. In both cases, the angiostatin, endostatin, or angiostatin-endostatin fusion DNA inserts were under the transcriptional control of the retroviral left Long Terminal Repeat (LTR). In the first vector, the dominant selectable marker was the Neomycin phosphotransferase gene (NeoR), which confers resistance to G418, and is driven by an internal phosphoglycerate kinase (PGK) promoter. In the second vector, the dominant selectable marker was the humanized, red-shifted green fluorescent protein (EGFP), which is co-translationally expressed by means of an Internal Ribosome Entry Site (IRES) from the Encephalomyocarditis virus (EMCV).

The retroviral gene therapy vectors were transfected by $CaPO_4$ precipitation in the transient ecotropic packaging cell-line BOSC 23, Pear et al., *PNAS* 90:8392 (1993). Viral supernatants were collected two days thereafter and filtered through 0.45 mm filters. Filtered viral supernatants were subsequently used to infect GENETIX's stable amphotropic retroviral packaging cell-line AM12 (Genetix Pharmaceuticals, Inc., Cambridge, MA). After another two days, viral supernatants from transduced AM12 were filtered

and used to infect GENETIX's stable ecotropic retroviral packaging cell-line GP+E86 (Genetix Pharmaceuticals, Inc.). Both transduced AM12 and GP+E86 were then selected in the presence of G418 (in the case of constructs bearing NeoR) or sorted by Fluorescent Activated Cell Sorter (FACS) for EGFP expression. Viral titers were estimated according to standard practice by counting G418 resistant colonies among NIH3T3 cells exposed to diluted virus preparation. Ecotropic viral titers were above 5 x $10^5$ /ml of viral supernatants, only 3-fold lower than "empty" control vectors. No Replication Competent Retrovirus (RCR) was detected in standard assays.

Example 3: Transduction of Target Cells Using Retroviral Gene Therapy Vectors

This example illustrates the stability of retroviral gene therapy vector transmission and the lack of toxicity in non-endothelial target cells.

Following 24-hour incubation of confluent viral producer cells in 100 mm plates, viral supernatant was removed and filtered (0.45 μm filter, Gelman Sciences, Ann Arbor, MI). Viral supernatant, containing 7 μg/ml polybrene (Sigma, St. Louis, MO), was added to target cells 24 hours after plating the target cells. Fresh medium was added after 4-12 hours, and, after an additional 48 hours, cells were selected for retroviral infection by exposure to medium containing 1 mg/ml G418 (Gibco BRL, Grand Island, NY) or by FACS sorting (FACStar cell sorter, Becton Dickinson, San Jose, CA). The stability of transmission of the retroviral gene therapy vectors described in Example 2 was examined by Southern blot analysis of transduced NIH3T3 cells, using specific probes (EGFP) and restriction enzyme digestion of genomic DNA with Sac1, which cuts only once in each LTR. Stable chromosomal integration of intact proviruses of appropriate length was observed with all constructs.

The lack of non-specific toxicity on non-endothelial cells was

established by using filtered viral supernatants to transduce various tumor cell-types and cell-lines (NIH3T3 cells, K562 cells (ATCC), and human SK-N-AS neuroblastoma cells; Cohen, P.S., *Cancer Research*, 55:2380 (1995). Transduced cell populations were subsequently selected with G418 or sorted for EGFP expression by FACS. No obvious effects on cell viability, growth or other phenotypical characteristics were detected.

Example 4: Protein Expression of Angiostatin, Endostatin and Angiostatin-Endostatin Fusion Proteins in Cells Transduced with Retroviral Gene Therapy Vectors

This example illustrates that recombinant angiostatin, endostatin, and angiostatin-endostatin fusion proteins were readily detected in retrovirally transduced cells and their supernatant, indicating efficient expression and secretion.

MSCV virus based vectors containing sequences encoding murine Kringle 1 (K1), K1K5, K1K2K3, K1K2K3K4, and K1K2K3K4K5 were used to transduce NIH3T3 cells. With regard to the murine recombinant proteins, Western blot analysis of transduced cells and their supernatant was performed by means of a monoclonal antibody that recognizes the FLAG polypeptide and/or export signal. Because this antibody is not mono-specific, significant cross-reactivity with murine proteins was apparent. However, by comparing the pattern obtained with mock cells, it was clear that the antibody revealed an additional band of appropriate size in all transduced cells. Moreover, the recombinant proteins were detected in cell supernatants at levels above 50 ng/ml, using a protein concentration/semi-purification procedure (Centricon columns, Amicon, Beverly, MA). With regard to the human recombinant proteins, no FLAG tag was added, so a monoclonal antibody that recognizes specifically the first three kringles of human plasminogen in its native, non-

denatured form was used: O'Reilly et al., *Cell* 79:315 (1994). Because of this constraint, Western blot analysis using denaturing gels could not be performed. An ELISA assay was performed which indicated that human recombinant angiostatin was detected at levels likely to be therapeutic according to previous findings in the model of Lewis Lung Carcinoma *Id.*

These results indicate that high levels of recombinant proteins of expected length were expressed in retrovirally transduced cells and were efficiently secreted.

Example 5: In Vivo Anti-Tumor Activity of Cells Transduced with Gene Therapy Vectors Encoding the Angiostatin-Endostatin Fusion Protein

Human SK-N-AS neuroblastoma cells (Cohen, 1995) were transduced with the retroviral gene therapy vector containing the angiostatin-endostatin fusion protein, described in Example 2. These cells (1,000,000) were suspended in 1 mL Dulbecco's phosphate buffered saline and injected into the right mid-quadrant of nude immuno-compromised mice. While no impairment of the in vitro growth of transduced cells was observed, a dramatic decrease in tumor growth in nude mice cells following subcutaneous implantation of the transduced cells was evident as compared to "mock virus"-transduced control cells.

Example 6: Ex Vivo Transfer of Retroviral Gene Therapy Vectors Encoding Anti-Angiogenic Polypeptides to Primary Hematopoietic Cells, and Subsequent Transplantation to Recipient Mice

This example illustrates infection of primary hematopoietic cells from

donor mice with retroviral gene therapy vectors encoding angiostatin, endostatin, or an angiostatin-endostatin fusion protein, and a selectable GFP marker, and subsequent transplantation of the transduced hematopoietic cells into recipient mice.

Femoral bone marrow cells are harvested from male donor C57BL6/J-Ly5.1 mice (Jackson Labs, Bar Harbor, ME), intravenously injected four days previously with 150 mg/kg of 5-fluorouracil (5-FU). Bone marrow cells are cultured for two days in medium composed of DMEM, 15% fetal calf serum, 10 ng/ml human IL-6, 6 ng/ml murine IL-3 and 100 ng/ml murine Steel factor prior to two days of culture atop a confluent monolayer of irradiated (1,500 cGy, $^{137}Cs$ $\gamma$-irradiation) viral producer cells in the above medium including 6 ug/ml of prolamine sulfate. The viral producer cells are transfected with a retroviral gene therapy vector, as described above. Upon completion of the co-culture infection protocol, recovered non-adherent cells are cultured for an additional 48 hours to allow for expression of the transferred GFP gene. Retrovirally transduced cells expressing the transferred GFP gene are subsequently identified and selected for, using a FACStar+ cell sorter (Becton Dickinson, San Jose, CA). The GFP+ cells are intravenously injected into congenic female C57BL6/J-Ly5.2 recipient mice (National Cancer Institute, Washington, DC) previously given 950 cGy (83cGy/min, $^{137}Cs$ $\gamma$-rays) of whole body irradiation. In each case, a small fraction of GFP+ sorted cells is used for day 12 CFU-S and in vitro clonogenic progenitor assays to assess the efficiency of the infection and selection procedures on these more mature cell types.

Example 7: Engraftment of Recipient Mice with Donor-Derived Hematopoietic Cells

This example illustrates engraftment of the recipient mice with the

donor-derived transfected hematopoietic cells from Example 6.

The donor and recipient mice are phenotypically distinguishable on the basis of Y chromosome specific sequences, as well as on the basis of allelic differences at the murine CD45 cell surface antigen locus. Male donor mice are homozygous for the CD45.2 allele, while female recipient mice are homozygous for CD45.1. The engraftment of recipient mice with donor-derived (CD45.2+) cells is assessed at both short (5 weeks) and long (34 months) time points post-transplant by flow cytometric analysis of peripheral blood samples stained with a phycoerythrin labeled antibody specific for the CD45.2 antigen (Pharmingen, San Diego, CA). The results indicate that engraftment occurs.

Example 8: Proviral Marking and GFP Expression in Recipient Mice

This example illustrates the presence of recombinant provirus and expression of the transferred GFP gene in the recipient mice from Example 6.

The level of proviral marking in reconstituted animals is initially determined by Southern blot and semi-quantitative PCR analysis of DNA obtained from peripheral blood leukocytes. The large majority of donor-derived (CD45.2+) cells in recipient mice contain a minimum of one copy of recombinant provirus. In addition, flow cytometric analysis of peripheral blood leukocytes is performed to ascertain the proportion of cells expressing the transferred GFP cDNA. Because the GFP and angiogenic inhibitor protein cDNAs are both driven from the same regulatory sequences, due to the inclusion of an internal ribosomal entry site (IRES) element, the analysis of GFP expression in the peripheral blood provides an indirect measurement of the levels of anti-angiogenic protein being expressed. The results indicate expression of the transferred genes.

Example 9: Anti-Angiogenic Polypeptide Expression in Recipient Mice

This example illustrates the presence of anti-angiogenic polypeptide in the sera of the recipient mice from Example 6, using both physical and functional assays.

Serum obtained from the transplanted animals described in Example 6 is used for ELISA using an antibody specific for the synthetic FLAG epitope (IBI, Eastman Kodak, New Haven, CT) and compared against known standards of purified protein. Results indicate the presence of the anti-angiogenic polypeptide in the serum.

To determine whether a functional anti-angiogenesis polypeptide is present in the circulation, sera from transplanted animals is tested for its ability to inhibit the proliferation of bovine capillary cells *in vitro;* O'Reilly (1994). Briefly, cells are plated in 24 well dishes at 25,000 cells/ml and maintained in DMEM with 5% bovine calf serum for 24 hours. The medium is then replaced with fresh medium containing various dilutions of the test serum. After 20 minutes of incubation, fresh medium including b-FGF (final concentration 1 ng/ml) is added and the cells are cultured for 72 hours. Cells are then dispersed using trypsin and the cell number determined by Coulter counter. Results indicate that functional anti-angiogenic polypeptide is present in the sera of the recipient mice.

In addition, the ability of circulating anti-angiogenic polypeptide to inhibit the growth of primary tumor cells is assessed. Transplanted mice are subcutaneously injected with one million Lewis lung carcinoma (LLC) cells (O'Reilly, (1994)) at the proximal midline of their dorsal skin. The mice are closely monitored for survival, tumor size and growth, and overall health. Results indicate that the anti-angiogenic polypeptides from the sera of the

recipient mice inhibit growth of the LLC tumor cells.

Finally, upon sacrifice of the transplanted recipient mice, blood, spleen, thymus and bone marrow are harvested and analyzed for the presence of proviral DNA by Southern analysis as well as expression of the transferred GFP and anti-angiogenic polypeptide cDNAs by flow cytometry and ELISA. Moreover, a portion of bone marrow cells is re-transplanted into secondary recipients to generate individual day 12 spleen colonies, as well as plated in methylcellulose to assess in vitro clonogenic progenitors. Individual clones are analyzed for proviral DNA by PCR or Southern blot, and for gene expression by flow cytometry and ELISA. Results of these tests also indicate the presence of proviral DNA and expression of the anti-angiogenic polypeptides and marker proteins.

Example 10: Evaluating the Efficacy of Retroviral Gene Therapy Vectors Encoding Anti-Angiogenic Polypetides on Various Human Cancers Implanted in SCID Mice Using Ex Vivo Gene Therapy

This example illustrates a method for rapidly screening various forms of human cancer to determine susceptibility to treatment by the systemic delivery of anti-angiogenic polypeptides.

The methods for gene transfer, assessment of proviral marking and assessment of transferred gene expression as described in Examples 3 through 9 are repeated using immuno-deficient SCID mice, with the following exceptions. Since SCID mice are more sensitive to $\gamma$-irradiation than C57BL6/J mice, the female SCID recipients receive a lower dose of 400cGy of whole body irradiation in contrast to the 950cGy required for C57BL6/J. In addition, since the SCID mice do not possess allelic differences at the CD45 cell surface antigen locus, donor and recipient cells are phenotypically distinguished on the basis of Y chromosome specific sequences using Southern

blot analysis.

Bone marrow from male donor SCID mice is infected, selected for on the basis of expression of the transferred GFP marker cDNA, and transplanted into irradiated female SCID recipients. Engraftment with provirally marked cells and expression of the transferred genes is demonstrated. The mice are then separately implanted with a variety of human tumor cell types, e.g., breast adenocarcinoma, lung squamous cell carcinoma, and brain glioblastoma. In each case, the ability of the anti-angiogenic polypeptides to inhibit the growth of the various human tumor cell types is monitored and quantified.

Example 11: Evaluating the Efficacy of Retroviral Gene Therapy Vectors Encoding Anti-Angiogenic Polypeptides for Treatment of Ovarian Cancer Using In Vivo Gene Therapy

This example illustrates the feasibility of using retroviral gene therapy vectors encoding anti-angiogenic polypeptides to achieve efficient gene transfer to established tumors in vivo using a well-established murine model of human ovarian cancer. Following injections, mice are closely monitored for tumor growth and survival.

Eight to ten week old nude mice (Jackson Labs, Bar Harbor are injected intra-peritoneally with 1 x $10^7$ PA-1 cells, an ovarian cancer cell-line (ATCC), and followed until palpable tumors are identified. Viral supernatant for in vivo injection is prepared as follows: Viral producer cells are grown to confluence in DMEM with 10% bovine calf serum, and the medium is then changed. After 24 hours of incubation, the viral conditioned supernatant is filtered though a 0.45 um low protein binding filter, protamine sulfate is added to a final concentration of 6ug/ml. the solution is aliquoted into 2 ml volumes, and frozen at -80°C. Recipient mice receive three intraperitoneal injections of viral supernatant (2 mls per injection) in addition to the polycation, over a period of

36 hours. Control mice are injected with medium collected from confluent dishes of NIH3T3 cells. Following injection of the viral conditioned supernatant, the mice are analyzed for survival as well as tumor growth over time as compared to mock injected controls. Results indicate that treatment of the ovarian cancer occurs. At death, the tumors are removed, weighed, and the cells dissociated for DNA extraction for Southern blot analysis to detect recombinant provirus.

Those skilled in the art will be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

In other embodiments, the invention provides methods and compositions for treating diseases and processes that are mediated by angiogenesis including, but not limited to, hemangioma, solid tumors, leukemia, metastasis, telangiectasia, psoriasis, scleroderma, pyogenic granuloma, myocardial angiogenesis, plaque neovascularization, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, corneal diseases, rubeosis, neovascular glaucoma, diabetic retinopathy, retrolental fibroplasia, arthritis, diabetic neovascularization, macular degeneration, wound healing, peptic ulcer, *Helicobacter* related diseases, fractures, keloids, vasculogenesis, hematopoiesis, ovulation, menstruation, placentation, and cat scratch fever.

What is claimed is:

CLAIMS

1. Use of a nucleic acid molecule which expresses an anti-angiogenic polypeptide selected from the group consisting of human angiostatin, murine angiostatin, human endostatin, murine endostatin, and angiogenesis-inhibiting fragments thereof in the preparation of a medicament for inhibiting tumor growth in a human patient harboring a solid tumor, wherein expression of the anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells, thereby inhibiting its growth.

2. Use of a nucleic acid molecule which expresses an anti-angiogenic polypeptide of the formula A-B, wherein

A and B are polypeptide and/or export signal joined by a polypeptide and/or export signal bond;

A contains at least amino acids and comprises kringles 1, 2, and 3 of human or murine angiostatin; and

B contains at least amino acids and includes at least 75% of the amino acid sequence of human or murine endostatin in the preparation of a medicament for inhibiting tumor growth in a human patient harboring a solid tumor, wherein expression of the anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells, thereby inhibiting its growth.

3. The use of claim 2, wherein A further comprises kringle region 4 of

human or murine angiostatin.

4. The use of claim 2 or claim 3, wherein A further comprises kringle 5 of human or murine plasminogen.

5. The use of claim 1 or claim 2, wherein said nucleic acid molecule constitutes a portion of a viral vector.

6. The use of claim 1 or claim 2, wherein said nucleic acid molecule constitutesa portion of a plasmid.

7. The use of claim 6, wherein said plasmid is carried in a cell-free carrier so that the plasmid transfects living cells of the patient following plasmid administration, causing expression of the anti-angiogenesis polypeptide and/or export signal in the patient such that angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells is inhibited, causing inhibition of tumor growth.

8. The use of claim 6, wherein said plasmid has been transfected into animal cells *ex vivo*, wherein said animal cells express the anti-angiogenesis polypeptide to inhibit tumor-associated angiogenesis and tumor growth.

9. The use of claim 5, wherein said viral vector is carried in a cell-free carrier, so that the viral vector is incorporated into living cells of the patient following viral vector administration, causing expression of the anti-

angiogenesis polypeptide in the patient such that angiogenesis in the vicinity of the tumor and/or systemically by diffusion of the recombinant protein to the vascular compartment from secreting transduced cells is inhibited, causing inhibition of tumor growth.

10. The use of claim 5, wherein animal cells are infected with said viral vector *ex vivo* and then administered to said patient, wherein said animal cells express the anti-angiogenesis polypeptide to inhibit tumor-associated angiogenesis and tumor growth.

11. The use of claim 8, wherein said animal cells are human cells.

12. The use of claim 11, wherein said human cells are autologous.

13. The use of claim 11, wherein said human cells are allogeneic.

14. The use of claim 10, wherein said animal cells are human cells.

15. The use of claim 14, wherein said human cells are autologous.

16. The use of claim 14, wherein said human cells are allogeneic.

17. The use of claim 5, wherein said viral vector is a retroviral vector.

18. The use of claim 5, wherein said viral vector is a non-retroviral vector selected from the group consisting of adenoviral, adeno-associated, herpes, Simliki  Forest virus, and poxvirus vectors.

19. The use of claim 17, wherein said retroviral vector is Murine Stem Cell Virus or a lentivirus.

20. The use of claim 1, wherein said angiostatin comprises kringles 1, 2 and 3.

21. The use of claim 20, wherein said angiostatin further comprises kringle 4.

22. The use of claim 1, wherein said anti-angiogenic polypeptide is a fusion of angiostatin or a biologically active fragment thereof and endostatin or a biologically active fragment thereof.

23. The use of claim 1, wherein said nucleic acid molecule includes a nucleotide sequence encoding a signal polypeptide and/or export signal for effecting secretion of said anti-angiogenesis polypeptide.

24. The use of claim 23, wherein said signal polypeptide and/or export signal is plasminogen signal polypeptide and/or export signal.

25. The use of claim 1, wherein said nucleic acid molecule includes a nucleotide sequence encoding a preactivation polypeptide and/or export signal for effecting Golgi and/or ER export of the anti-angiogenic polypeptide..

26. The use of claim 25, wherein said preactivation polypeptide and/or export signal is a preactivation polypeptide and/or export signal of a human protein of the blood coagulation cascade.

27. The use of claim 26, wherein said preactivation polypeptide and/or export signal is human plasminogen preactivation polypeptide and/or export signal.

28. The method of claim 25, wherein the preactivation encoding sequence is positioned between a signal-encoding sequence and the sequence encoding the anti-angiogenic polypeptide and/or export signal.

29. The use of claim 1, wherein said nucleic acid molecule includes a nucleotide sequence encoding a tag for identification of said anti-angiogenic polypeptide.

30. The method of claim 27, wherein said tag is a Flag tag polypeptide and/or export signal.

31. A viral gene therapy vector comprising a nucleic acid molecule which encodes an anti-angiogenic polypeptide selected from the group consisting of human angiostatin, murine angiostatin, human endostatin, murine endostatin, and angiogenesis-inhibiting fusions and fragments thereof, wherein said viral vector is sufficiently attenuated for use in human gene therapy.

32. A human cell infected with the vector of claim 31.

33. Use of a nucleic acid molecule which expresses in said patient an anti-angiogenic polypeptide selected from the group consisting of human angiostatin, murine angiostatin, human endostatin, murine endostatin, and angiogenesis-inhibiting fusions and fragments thereof, in the preparation of a

medicament for treating a human patient suffering from diabetic retinopathy, wherein expression of the anti-angiogenic polypeptide in the patient inhibits angiogenesis in the vicinity of the retina.

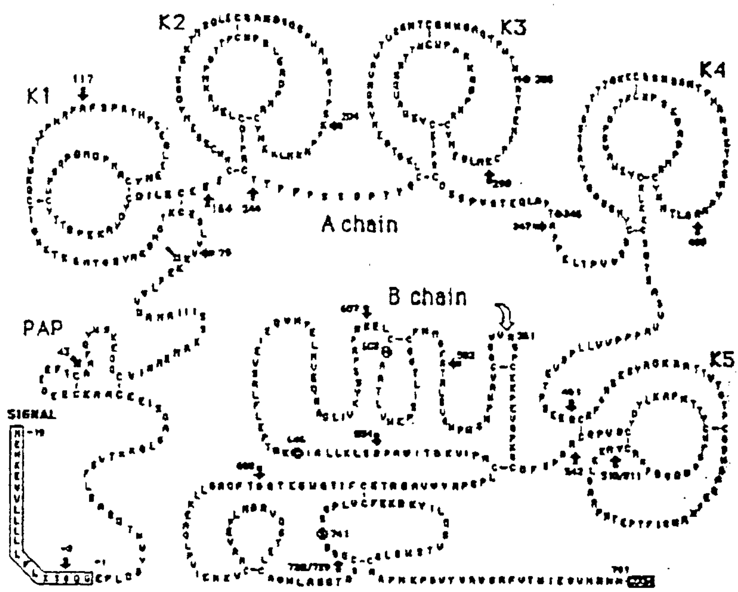


FIG. 1

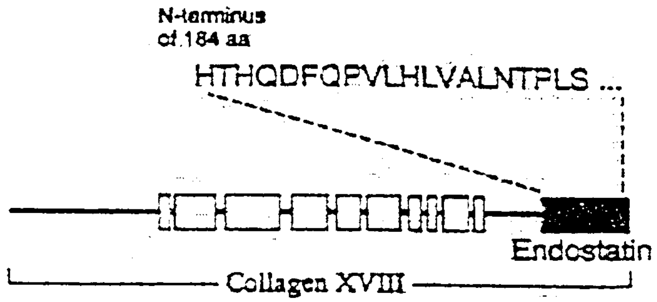
N-terminus
cf.184 aa
HTHQDFQPVLHLVALNTPLS ...
Endostatin
Collagen XVIII

Fig. 2

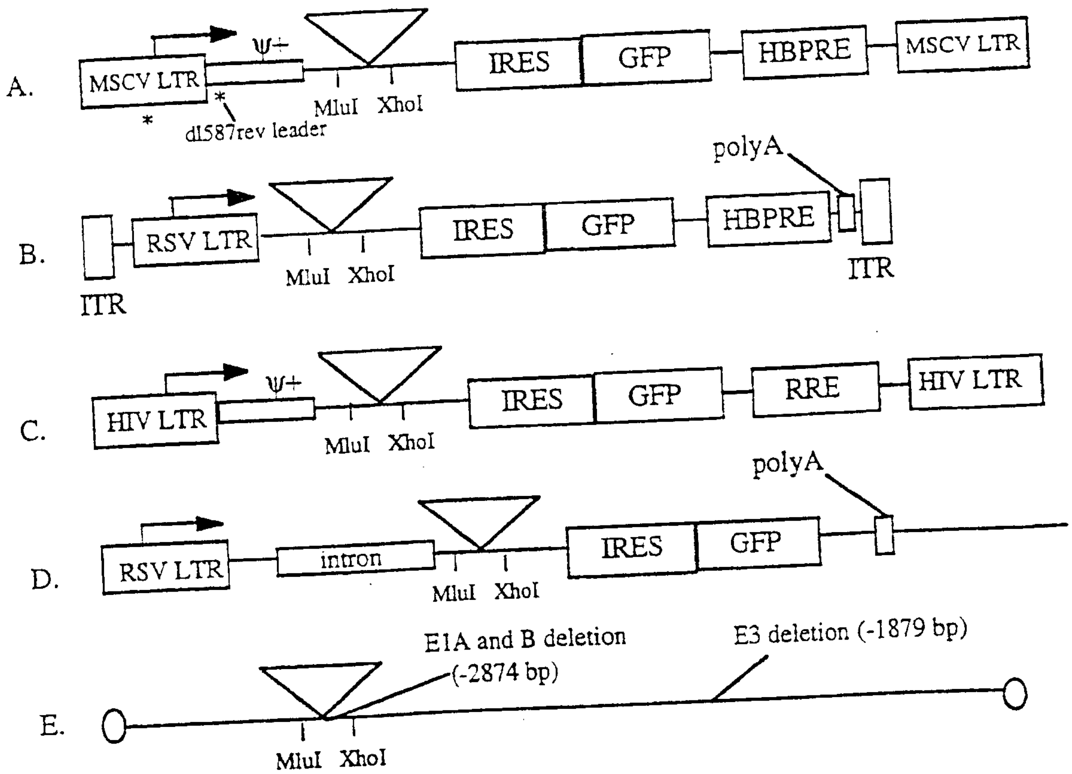
A.
MSCV LTR
Ψ+
*
*
dl587rev leader
MluI
XhoI
IRES
GFP
HBPRE
MSCV LTR
B.
ITR
RSV LTR
MluI
XhoI
IRES
GFP
HBPRE
polyA
ITR
C.
HIV LTR
Ψ+
MluI
XhoI
IRES
GFP
RRE
HIV LTR
D.
RSV LTR
intron
MluI
XhoI
IRES
GFP
polyA
E.
E1A and B deletion
(-2874 bp)
E3 deletion (-1879 bp)
MluI
XhoI

FIG. 3

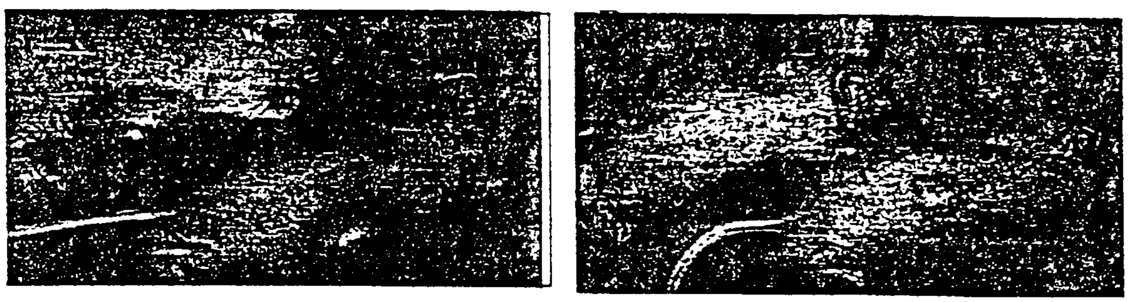

FIG. 4

human plasminogen

signal peptide preactivation peptide (PAP)

10 20 30 40 50 60 70 80 90 100
ATGGAACATAAGGAAGTGGTTCTTCTACTTCTTTTATTTCTGAAATCAGGTCAAGGAGAGCCTCTGGATGACTATGTGAATACCCAGGGGGCTTCACTGT
MetGluHisLysGluValValLeuLeuLeuLeuLeuPheLeuLysSerGlyGlnGlyGluProLeuAspAspTyrValAsnThrGlnGlyAlaSerLeuPhe

110 120 130 140 150 160 170 180 190 200
TCAGTGTCACTAAGAAGCAGCTGGGAGCAGGAAGTATAGAAGAATGTGCAGCAAAATGTGAGGAGGACGAAGAATTCACCTGCAGGGCATTCCAATATCA
SerValThrLysLysGlnLeuGlyAlaGlySerIleGluGluCysAlaAlaLysCysGluGluAspGluGluPheThrCysArgAlaPheGlnTyrHis

Kringle 1-3

210 220 230 240 250 260 270 280 290 300
CAGTAAAGAGCAACAATGTGTGATAATGGCTGAAAACAGGAAGTCCTCCATAATCATTAGGATGAGAGATGTAGTTTTATTTGAAAAGAAAGTGTATCTC
SerLysGluGlnGlnCysValIleMetAlaGluAsnArgLysSerSerIleIleIleArgMetArgAspValValLeuPheGluLysLysValTyrLeu

310 320 330 340 350 360 370 380 390 400
TCAGAGTGCAAGACTGGGAATGGAAAGAACTACAGAGGGACGATGTCCAAAACAAAAAATGGCATCACCTGTCAAAAATGGAGTTCCACTTCTCCCCACA
SerGluCysLysThrGlyAsnGlyLysAsnTyrArgGlyThrMetSerLysThrLysAsnGlyIleThrCysGlnLysTrpSerSerThrSerProHisArg

410 420 430 440 450 460 470 480 490 500
GACCTAGATTCTCACCTGCTACACACCCCTCAGAGGGACTGGAGGAGAACTACTGCAGGAATCCAGACAACGATCCGCAGGGGCCCTGGTGCTATACTAC
ProArgPheSerProAlaThrHisProSerGluGlyLeuGluGluAsnTyrCysArgAsnProAspAsnAspProGlnGlyProTrpCysTyrThrThr

510 520 530 540 550 560 570 580 590 600
TGATCCAGAAAAGAGATATGACTACTGCGACATTCTTGAGTGTGAAGAGGAATGTATGCATTGCAGTGGAGAAAACTATGACGGCAAAATTTCCAAGACC
AspProGluLysArgTyrAspTyrCysAspIleLeuGluCysGluGluGluCysMetHisCysSerGlyGluAsnTyrAspGlyLysIleSerLysThr

610 620 630 640 650 660 670 680 690 700
ATGTCTGGACTGGAATGCCAGGCCTGGGACTCTCAGAGCCCACACGCTCATGGATACATTCCTTCCAAATTTCCAAACAAGAACCTGAAGAAGAATTACT
MetSerGlyLeuGluCysGlnAlaTrpAspSerGlnSerProHisAlaHisGlyTyrIleProSerLysPheProAsnLysAsnLeuLysLysAsnTyrCys

710 720 730 740 750 760 770 780 790 800
GTCGTAACCCCGATAGGGAGCTGCGGCCTTGGTGTTTCACCACCGACCCCAACAAGCGCTGGGAACTTTGCGACATCCCCCGCTGCACAACACCTCCACC
ArgAsnProAspArgGluLeuArgProTrpCysPheThrThrAspProAsnLysArgTrpGluLeuCysAspIleProArgCysThrThrProProPro

810 820 830 840 850 860 870 880 890 900
ATCTTCTGGTCCCACCTACCAGTGTCTGAAGGGAACAGGTGAAAACTATCGCGGGAATGTGGCTGTTACCGTTTCCGGGCACACCTGTCAGCACTGGAGT
SerSerGlyProThrTyrGlnCysLeuLysGlyThrGlyGluAsnTyrArgGlyAsnValAlaValThrValSerGlyHisThrCysGlnHisTrpSer

910 920 930 940 950 960 970 980 990 1000
GCACAGACCCCTCACACACATAACAGGACACCAGAAAACTTCCCCTGCAAAAATTTGGATGAAAACTACTGCCGCAATCCTGACGGAAAAAGGGCCCCAT
AlaGlnThrProHisThrHisAsnArgThrProGluAsnPheProCysLysAsnLeuAspGluAsnTyrCysArgAsnProAspGlyLysArgAlaProTrp

K1-3 K4

1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
GGTGCCATACAACCAACAGCCAAGTGCGGTGGGAGTACTGTAAGATACCGTCCTGTGACTCCTCCCCAGTATCCACGGAACAATTGGCTCCCACAGCACC
CysHisThrThrAsnSerGlnValArgTrpGluTyrCysLysIleProSerCysAspSerSerProValSerThrGluGlnLeuAlaProThrAlaPro

1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
ACCTGAGCTAACCCCTGTGGTCCAGGACTGCTACCATGGTGATGGACAGAGCTACCGAGGCACATCCTCCACCACCACCACAGGAAAGAAGTGTCAGTCT
ProGluLeuThrProValValGlnAspCysTyrHisGlyAspGlyGlnSerTyrArgGlyThrSerSerThrThrThrThrGlyLysLysCysGlnSer

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
TGGTCATCTATGACACCACACCGGCACCAGAAGACCCCAGAAAACTACCCAAATGCTGGCCTGACAATGAACTACTGCAGGAATCCAGATGCCGATAAAG
TrpSerSerMetThrProHisArgHisGlnLysThrProGluAsnTyrProAsnAlaGlyLeuThrMetAsnTyrCysArgAsnProAspAlaAspLysGly

K4 K5

1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
GCCCCTGGTGTTTTACCACAGACCCCAGCGTCAGGTGGGAGTACTGCAACCTGAAAAAATGCTCAGGAACAGAAGCGAGTGTTGTAGCACCTCCGCCTGT
ProTrpCysPheThrThrAspProSerValArgTrpGluTyrCysAsnLeuLysLysCysSerGlyThrGluAlaSerValValAlaProProProVal

1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
TGTCCTGCTTCCAGATGTAGAGACTCCTTCCGAAGAAGACTGTATGTTTGGGAATGGAAAGGATACCGAGGCAAGAGGGCGACCACTGTTACTGGGACG
ValLeuLeuProAspValGluThrProSerGluGluAspCysMetPheGlyAsnGlyLysGlyTyrArgGlyLysArgAlaThrThrValThrGlyThr

1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
CCATGCCAGGACTGGGCTGCCCAGGAGCCCCATAGACACAGCATTTTCACTCCAGAGACAAATCCACGGGCGGGTCTGGAAAAAAATTACTGCCGTAACC
ProCysGlnAspTrpAlaAlaGlnGluProHisArgHisSerIlePheThrProGluThrAsnProArgAlaGlyLeuGluLysAsnTyrCysArgAsnPro

1610 1620 1630 1640 1650 1660 1670 1680 1690 1700
CTGATGGTGATGTAGGTGGTCCCTGGTGCTACACGACAAATCCAAGAAAACTTTACGACTACTGTGATGTCCCTCAGTGTGCGGCCCCTTCATTTGATTG
AspGlyAspValGlyGlyProTrpCysTyrThrThrAsnProArgLysLeuTyrAspTyrCysAspValProGlnCysAlaAlaProSerPheAspCys

K5

1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
TGGGAAGCCTCAAGTGGAGCCGAAGAAATGTCCTGGAAGGGTTGTGGGGGGGTGTGTGGCCCACCCACATTCCTGGCCCTGGCAAGTCAGTCTTAGAACA
GlyLysProGlnValGluProLysLysCysProGlyArgValValGlyGlyCysValAlaHisProHisSerTrpProTrpGlnValSerLeuArgThr

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900
AGGTTTGGAATGCACTTCTGTGGAGGCACCTTGATATCCCCAGAGTGGGTGTTGACTGCTGCCCACTGCTTGGAGAAGTCCCCAAGGCCTTCATCCTACA
ArgPheGlyMetHisPheCysGlyGlyThrLeuIleSerProGluTrpValLeuThrAlaAlaHisCysLeuGluLysSerProArgProSerSerTyrLys

1910 1920 1930 1940 1950 1960 1970 1980 1990 2000
AGGTCATCCTGGGTGCACACCAAGAAGTGAATCTCGAACCGCATGTTCAGGAAATAGAAGTGTCTAGGCTGTTCTTGGAGCCCACACGAAAAGATATTGC
ValIleLeuGlyAlaHisGlnGluValAsnLeuGluProHisValGlnGluIleGluValSerArgLeuPheLeuGluProThrArgLysAspIleAla

2010 2020 2030 2040 2050 2060 2070 2080 2090 2100
CTTGCTAAAGCTAAGCAGTCCTGCCGTCATCACTGACAAAGTAATCCCAGCTTGTCTGCCATCCCCAAATTATGTGGTCGCTGACCGGACCGAATGTTTC
LeuLeuLysLeuSerSerProAlaValIleThrAspLysValIleProAlaCysLeuProSerProAsnTyrValValAlaAspArgThrGluCysPhe

2110 2120 2130 2140 2150 2160 2170 2180 2190 2200
ATCACTGGCTGGGGAGAAACCCAAGGTACTTTTGGAGCTGGCCTTCTCAAGGAAGCCCAGCTCCCTGTGATTGAGAATAAAGTGTGCAATCGCTATGAGT
IleThrGlyTrpGlyGluThrGlnGlyThrPheGlyAlaGlyLeuLeuLysGluAlaGlnLeuProValIleGluAsnLysValCysAsnArgTyrGluPhe

2210 2220 2230 2240 2250 2260 2270 2280 2290 2300
TTCTGAATGGAAGAGTCCAATCCACCGAACTCTGTGCTGGGCATTTGGCCGGAGGCACTGACAGTTGCCAGGGTGACAGTGGAGGTCCTCTGGTTTGCTT
LeuAsnGlyArgValGlnSerThrGluLeuCysAlaGlyHisLeuAlaGlyGlyThrAspSerCysGlnGlyAspSerGlyGlyProLeuValCysPhe

2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
CGAGAAGGACAAATACATTTTACAAGGAGTCACTTCTTGGGGTCTTGGCTGTGCACGCCCCAATAAGCCTGGTGTCTATGTTCGTGTTTCAAGGTTTGTT
GluLysAspLysTyrIleLeuGlnGlyValThrSerTrpGlyLeuGlyCysAlaArgProAsnLysProGlyValTyrValArgValSerArgPheVal

2410 2420 2430 2440
ACTTGGATTGAGGGAGTGATGAGAAATAATTAA
ThrTrpIleGluGlyValMetArgAsnAsn

end human plasminogen coding sequence.

Fig. 5

start mouse endostatin

mouse endostatin

```
        10        20        30        40        50        60        70        80        90       100
CATACTCATCAGGACTTTCAGCCAGTGCTCCACCTGGTGGCACTGAACACCCCCCTGTCTGGAGGCATGCGTGGTATCCGTGGAGCAGATTTCCAGTGCT
HisThrHisGlnAspPheGlnProValLeuHisLeuValAlaLeuAsnThrProLeuSerGlyGlyMetArgGlyIleArgGlyAlaAspPheGlnCysPhe

       110       120       130       140       150       160       170       180       190       200
TCCAGCAAGCCCGAGCCGTGGGGCTGTCGGGCACCTTCCGGGCTTTCCTGTCCTCTAGGCTGCAGGATCTCTATAGCATCGTGCGCCGTGCTGACCGGGG
  GlnGlnAlaArgAlaValGlyLeuSerGlyThrPheArgAlaPheLeuSerSerArgLeuGlnAspLeuTyrSerIleValArgArgAlaAspArgGly

       210       220       230       240       250       260       270       280       290       300
GTCTGTGCCCATCGTCAACCTGAAGGACGAGGTGCTATCTCCCAGCTGGGACTCCCTGTTTTCTGGCTCCCAGGGTCAAGTGCAACCCGGGGCCCGCATC
 SerValProIleValAsnLeuLysAspGluValLeuSerProSerTrpAspSerLeuPheSerGlySerGlnGlyGlnValGlnProGlyAlaArgIle

       310       320       330       340       350       360       370       380       390       400
TTTTCTTTTGACGGCAGAGATGTCCTGAGACACCCAGCCTGGCCGCAGAAGAGCGTATGGCACGGCTCGGACCCCAGTGGGCGGAGGCTGATGGAGAGTT
PheSerPheAspGlyArgAspValLeuArgHisProAlaTrpProGlnLysSerValTrpHisGlySerAspProSerGlyArgArgLeuMetGluSerTyr

       410       420       430       440       450       460       470       480       490       500
ACTGTGAGACATGGCGAACTGAAACTACTGGGGCTACAGGTCAGGCCTCCTCCCTGCTGTCAGGCAGGCTCTTGGAACAGAAAGCTGCGAGCTGCCACAA
  CysGluThrTrpArgThrGluThrThrGlyAlaThrGlyGlnAlaSerSerLeuLeuSerGlyArgLeuLeuGluGlnLysAlaAlaSerCysHisAsn

       510       520       530       540       550       560       570       580       590       600
CAGCTACATCGTCCTCTGCATTGAGAATAGCTTCATGACCTCTTTCTCCAAATAGGCCTCTGCCAGCTAGGGTGGCAGACAGAGGCCATGCAGAACTTTG
 SerTyrIleValLeuCysIleGluAsnSerPheMetThrSerPheSerLysEndAlaSerAlaSerEndGlyGlyArgGlnArgProCysArgThrLeu

       610       620       630       640       650       660       670       680       690       700
ACACAGCGCAGGGAGCATTCAGTCAGCACCCAGGGCTCTGGCTGGGATACAACTCCTGTATAGTTCCCATTTTTATGTAATCCTCAAGAAATAAAAGGAA
ThrGlnArgArgGluHisSerValSerThrGlnGlySerGlyTrpAspThrThrProValEndPheProPheLeuCysAsnProGlnGluIleLysGlySer

       710       720
GCCAAAGAGTAAAAAAAAAA
  GlnArgValLysLys
```

end mouse endostatin coding sequence

Fig 6

Mouse Plasminogen cDNA

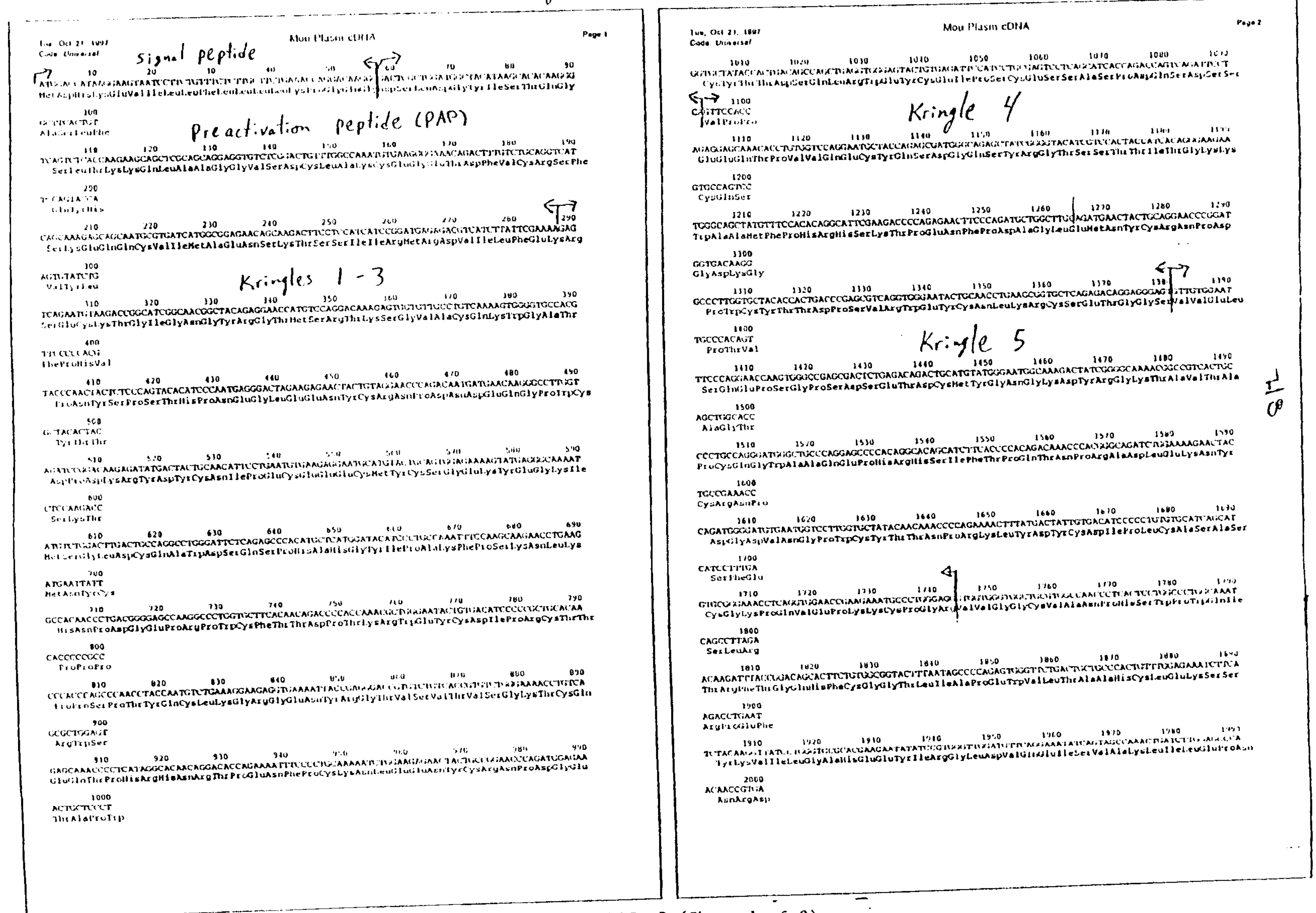


FIG. 7 (Sheet 1 of 2)

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 IleAlaLeuLeuLysLeuSerArgProAlaThrIleThrAspLysValIleProAlaCysLeuProSerProAsnTyrMetValAlaAsp

       2100
CCGGACAATA
 ArgThrIle

       2110      2120      2130      2140      2150      2160      2170      2180      2190
TGTTACATCACCGGCTGGGGAGAGACTCAAGGGACTTTCGGTGCCGGTCGTCTCAAGGAGGCTCAGCTGCCTGTGATTGAGAACAAGGTG
CysTyrIleThrGlyTrpGlyGluThrGlnGlyThrPheGlyAlaGlyArgLeuLysGluAlaGlnLeuProValIleGluAsnLysVal

       2200
TGCAACCGCG
CysAsnArgVal

       2210      2220      2230      2240      2250      2260      2270      2280      2290
TCGAGTATCTGAACAACAGAGTCAAATCCACGGAGCTCTGTGCCGGGCAACTGGCTGGTGGCGTCGACAGCTGCCAAGGCGACAGTGGAG
  GluTyrLeuAsnAsnArgValLysSerThrGluLeuCysAlaGlyGlnLeuAlaGlyGlyValAspSerCysGlnGlyAspSerGlyGly

       2300
GACCTCTGGT
  ProLeuVal

       2310      2320      2330      2340      2350      2360      2370      2380      2390
TTGCTTCGAGAAGGACAAGTACATTTTACAAGGAGTCACTTCTTGGGGTCTTGGCTGTGCTCGCCCCAATAAGCCTGGTGTCTACGTTCG
 CysPheGluLysAspLysTyrIleLeuGlnGlyValThrSerTrpGlyLeuGlyCysAlaArgProAsnLysProGlyValTyrValArg

       2400
TGTCTCACGG
 ValSerArg
```

end plasminogen coding sequence.

```
       2410      2420      2430      2440      2450      2460      2470      2480      2490
TTTGTTGATTGGATTGAAAGGGAGATGAGGAATAACTGACTAGGTGGAAGGCCGAGCAAAACCTCTGCTTACTAAAGCTTACTGAATATG
PheValAspTrpIleGluArgGluMetArgAsnAsnEndLeuGlyGlyArgProSerLysThrSerAlaTyrEndSerLeuLeuAsnMet

       2500
GGGAGAGGGC
GlyArgGlyLeu

       2510      2520      2530      2540      2550      2560      2570      2580      2590
TTAGGGTGTTTGGAAAAACTGACAGTAATCAAACTGGGACACTACACTGAACCACAGCTTCCTGTCGCCCCTCAGCCCCTCCCCTTTTTT
  ArgValPheGlyLysThrAspSerAsnGlnThrGlyThrLeuHisEndThrThrAlaSerCysArgProSerAlaProProLeuPheLeu

       2600
TGTATTATTG
  TyrTyrCys

       2610      2620      2630      2640      2650      2660      2670      2680      2690
TGGGTAAAATTTTCCTGTCTGTGGACTTCTGGATTTTGTGACAATAGACCATCACTGCTGTGACCTTTGTTGAAAATAAACTCGATACTT
 GlyEndAsnPheProValCysGlyLeuLeuAspPheValThrIleAspHisHisCysCysAspLeuCysEndLysEndThrArgTyrLeu

       2700
ACTTTG
 Leu
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FIG. 7 (Sheet 2 of 2

SEQUENCE LISTING

<110> Genetix Pharmaceuticals, Inc.

<120> ANTI-ANGIOGENIC GENE THERAPY VECTORS AND
THEIR USE IN TREATING ANGIOGENISIS-RELATED DISEASES

<130> 50033/002WO1

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 1               5                  10                  15

ggt caa gga gag cct ctg gat gac tat gtg aat acc cag ggg gct tca      96
Gly Gln Gly Glu Pro Leu Asp Asp Tyr Val Asn Thr Gln Gly Ala Ser
            20                  25                  30

ctg ttc agt gtc act aag aag cag ctg gga gca gga agt ata gaa gaa     144
Leu Phe Ser Val Thr Lys Lys Gln Leu Gly Ala Gly Ser Ile Glu Glu
        35                  40                  45

tgt gca gca aaa tgt gag gag gac gaa gaa ttc acc tgc agg gca ttc     192
Cys Ala Ala Lys Cys Glu Glu Asp Glu Glu Phe Thr Cys Arg Ala Phe
    50                  55                  60

caa tat cac agt aaa gag caa caa tgt gtg ata atg gct gaa aac agg     240
Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Arg
65                  70                  75                  80

aag tcc tcc ata atc att agg atg aga gat gta gtt tta ttt gaa aag     288
Lys Ser Ser Ile Ile Ile Arg Met Arg Asp Val Val Leu Phe Glu Lys
                85                  90                  95
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aaa gtg tat ctc tca gag tgc aag act ggg aat gga aag aac tac aga       336
Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg
            100                 105                 110

ggg acg atg tcc aaa aca aaa aat ggc atc acc tgt caa aaa tgg agt       384
Gly Thr Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser
        115                 120                 125

tcc act tct ccc cac aga cct aga ttc tca cct gct aca cac ccc tca       432
Ser Thr Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser
    130                 135                 140

gag gga ctg gag gag aac tac tgc agg aat cca gac aac gat ccg cag       480
Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln
145                 150                 155                 160

ggg ccc tgg tgc tat act act gat cca gaa aag aga tat gac tac tgc       528
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys
                165                 170                 175

gac att ctt gag tgt gaa gag gaa tgt atg cat tgc agt gga gaa aac       576
Asp Ile Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn
            180                 185                 190

tat gac ggc aaa att tcc aag acc atg tct gga ctg gaa tgc cag gcc       624
Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala
        195                 200                 205

tgg gac tct cag agc cca cac gct cat gga tac att cct tcc aaa ttt       672
Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe
    210                 215                 220

cca aac aag aac ctg aag aag aat tac tgt cgt aac ccc gat agg gag       720
Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Arg Glu
225                 230                 235                 240

ctg cgg cct tgg tgt ttc acc acc gac ccc aac aag cgc tgg gaa ctt       768
Leu Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu
                245                 250                 255

tgc gac atc ccc cgc tgc aca aca cct cca cca tct tct ggt ccc acc       816
Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr
            260                 265                 270

tac cag tgt ctg aag gga aca ggt gaa aac tat cgc ggg aat gtg gct       864
Tyr Gln Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala
        275                 280                 285

gtt acc gtt tcc ggg cac acc tgt cag cac tgg agt gca cag acc cct       912
Val Thr Val Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr Pro
    290                 295                 300

cac aca cat aac agg aca cca gaa aac ttc ccc tgc aaa aat ttg gat       960
His Thr His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp
305                 310                 315                 320
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gaa aac tac tgc cgc aat cct gac gga aaa agg gcc cca tgg tgc cat 1008
Glu Asn Tyr Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys His
325 330 335

aca acc aac agc caa gtg cgg tgg gag tac tgt aag ata ccg tcc tgt 1056
Thr Thr Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys
340 345 350

gac tcc tcc cca gta tcc acg gaa caa ttg gct ccc aca gca cca cct 1104
Asp Ser Ser Pro Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro
355 360 365

gag cta acc cct gtg gtc cag gac tgc tac cat ggt gat gga cag agc 1152
Glu Leu Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser
370 375 380

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385 390 395 400

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Trp Ser Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr
405 410 415

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420 425 430

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Lys Gly Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
435 440 445

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Cys Asn Leu Lys Lys Cys Ser Gly Thr Glu Ala Ser Val Val Ala Pro
450 455 460

ccg cct gtt gtc ctg ctt cca gat gta gag act cct tcc gaa gaa gac 1440
Pro Pro Val Val Leu Leu Pro Asp Val Glu Thr Pro Ser Glu Glu Asp
465 470 475 480

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Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg Gly Lys Arg Ala Thr Thr
485 490 495

gtt act ggg acg cca tgc cag gac tgg gct gcc cag gag ccc cat aga 1536
Val Thr Gly Thr Pro Cys Gln Asp Trp Ala Ala Gln Glu Pro His Arg
500 505 510

cac agc att ttc act cca gag aca aat cca cgg gcg ggt ctg gaa aaa 1584
His Ser Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu Lys
515 520 525

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Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Gly Gly Pro Trp Cys Tyr

530 535 540

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Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Val Pro Gln Cys
545 550 555 560

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Ala Ala Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys Lys
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35 40 45
Cys Ala Ala Lys Cys Glu Glu Asp Glu Glu Phe Thr Cys Arg Ala Phe
50 55 60
Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Arg
65 70 75 80
Lys Ser Ser Ile Ile Ile Arg Met Arg Asp Val Val Leu Phe Glu Lys
85 90 95
Lys Val Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg
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Gly Thr Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser
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Ser Thr Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser
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Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln
145 150 155 160
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys
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Asp Ile Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn
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Tyr Asp Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala
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Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe
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Pro Asn Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Arg Glu
225 230 235 240
Leu Arg Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu
245 250 255
Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr

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Tyr Gln Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala
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His Thr His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp
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Glu Asn Tyr Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys His
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Thr Thr Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys
340 345 350
Asp Ser Ser Pro Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro
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Glu Leu Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser
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Trp Ser Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr
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Pro Asn Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp
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Lys Gly Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
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Cys Asn Leu Lys Lys Cys Ser Gly Thr Glu Ala Ser Val Val Ala Pro
450 455 460
Pro Pro Val Val Leu Leu Pro Asp Val Glu Thr Pro Ser Glu Glu Asp
465 470 475 480
Cys Met Phe Gly Asn Gly Lys Gly Tyr Arg Gly Lys Arg Ala Thr Thr
485 490 495
Val Thr Gly Thr Pro Cys Gln Asp Trp Ala Ala Gln Glu Pro His Arg
500 505 510
His Ser Ile Phe Thr Pro Glu Thr Asn Pro Arg Ala Gly Leu Glu Lys
515 520 525
Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Gly Gly Pro Trp Cys Tyr
530 535 540
Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Val Pro Gln Cys
545 550 555 560
Ala Ala Pro Ser Phe Asp Cys Gly Lys Pro Gln Val Glu Pro Lys Lys
565 570 575
Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala His Pro His Ser Trp
580 585 590
Pro Trp Gln Val Ser Leu Arg Thr Arg Phe Gly Met His Phe Cys Gly
595 600 605
Gly Thr Leu Ile Ser Pro Glu Trp Val Leu Thr Ala Ala His Cys Leu
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Glu Lys Ser Pro Arg Pro Ser Ser Tyr Lys Val Ile Leu Gly Ala His
625 630 635 640
Gln Glu Val Asn Leu Glu Pro His Val Gln Glu Ile Glu Val Ser Arg
645 650 655
Leu Phe Leu Glu Pro Thr Arg Lys Asp Ile Ala Leu Leu Lys Leu Ser
660 665 670
Ser Pro Ala Val Ile Thr Asp Lys Val Ile Pro Ala Cys Leu Pro Ser
675 680 685
Pro Asn Tyr Val Val Ala Asp Arg Thr Glu Cys Phe Ile Thr Gly Trp
690 695 700

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Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Leu Leu Lys Glu Ala Gln
705                 710                 715                 720
Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Tyr Glu Phe Leu Asn
                725                 730                 735
Gly Arg Val Gln Ser Thr Glu Leu Cys Ala Gly His Leu Ala Gly Gly
            740                 745                 750
Thr Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys Phe Glu
        755                 760                 765
Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu Gly Cys
    770                 775                 780
Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg Phe Val
785                 790                 795                 800
Thr Trp Ile Glu Gly Val Met Arg Asn Asn
                805                 810


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<211> 2696
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(2436)

<400> 3
atg gac cat aag gaa gta atc ctt ctg ttt ctc ttg ctt ctg aaa cca      48
Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Leu Lys Pro
 1               5                  10                  15

gga caa ggg gac tcg ctg gat ggc tac ata agc aca caa ggg gct tca      96
Gly Gln Gly Asp Ser Leu Asp Gly Tyr Ile Ser Thr Gln Gly Ala Ser
            20                  25                  30

ctg ttc agt ctc acc aag aag cag ctc gca gca gga ggt gtc tcg gac     144
Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp
        35                  40                  45

tgt ttg gcc aaa tgt gaa ggg gaa aca gac ttt gtc tgc agg tca ttc     192
Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe
    50                  55                  60

cag tac cac agc aaa gag cag caa tgc gtg atc atg gcg gag aac agc     240
Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser
65                  70                  75                  80

aag act tcc tcc atc atc cgg atg aga gac gtc atc tta ttc gaa aag     288
Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys
                85                  90                  95

aga gtg tat ctg tca gaa tgt aag acc ggc atc ggc aac ggc tac aga     336
Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg
            100                 105                 110

gga acc atg tcc agg aca aag agt ggt gtt gcc tgt caa aag tgg ggt     384
Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly
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115 120 125

gcc acg ttc ccc cac gta ccc aac tac tct ccc agt aca cat ccc aat 432
Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn
130 135 140

gag gga cta gaa gag aac tac tgt agg aac cca gac aat gat gaa caa 480
Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln
145 150 155 160

ggg cct tgg tgc tac act aca gat ccg gac aag aga tat gac tac tgc 528
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys
165 170 175

aac att cct gaa tgt gaa gag gaa tgc atg tac tgc agt gga gaa aag 576
Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys
180 185 190

tat gag ggc aaa atc tcc aag acc atg tct gga ctt gac tgc cag gcc 624
Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala
195 200 205

tgg gat tct cag agc cca cat gct cat gga tac atc cct gcc aaa ttt 672
Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe
210 215 220

cca agc aag aac ctg aag atg aat tat tgc cac aac cct gac ggg gag 720
Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu
225 230 235 240

cca agg ccc tgg tgc ttc aca aca gac ccc acc aaa cgc tgg gaa tac 768
Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr
245 250 255

tgt gac atc ccc cgc tgc aca aca ccc ccg ccc cca ccc agc cca acc 816
Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr
260 265 270

tac caa tgt ctg aaa gga aga ggt gaa aat tac cga ggg acc gtg tct 864
Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser
275 280 285

gtc acc gtg tct ggg aaa acc tgt cag cgc tgg agt gag caa acc cct 912
Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro
290 295 300

cat agg cac aac agg aca cca gaa aat ttc ccc tgc aaa aat ctg gaa 960
His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu
305 310 315 320

gag aac tac tgc cgg aac cca gat gga gaa act gct ccc tgg tgc tat 1008
Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
325 330 335

acc act gac agc cag ctg agg tgg gag tac tgt gag att cca tcc tgc 1056

Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys
340 345 350

gag tcc tca gca tca cca gac cag tca gat tcc tca gtt cca cca gag 1104
Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu
355 360 365

gag caa aca cct gtg gtc cag gaa tgc tac cag agc gat ggg cag agc 1152
Glu Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser
370 375 380

tat cgg ggt aca tcg tcc act acc atc aca ggg aag aag tgc cag tcc 1200
Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser
385 390 395 400

tgg gca gct atg ttt cca cac agg cat tcg aag acc cca gag aac ttc 1248
Trp Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe
405 410 415

cca gat gct ggc ttg gag atg aac tac tgc agg aac ccg gat ggt gac 1296
Pro Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp
420 425 430

aag ggc cct tgg tgc tac acc act gac ccg agc gtc agg tgg gaa tac 1344
Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
435 440 445

tgc aac ctg aag cgg tgc tca gag aca gga ggg agt gtt gtg gaa ttg 1392
Cys Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly Ser Val Val Glu Leu
450 455 460

ccc aca gtt tcc cag gaa cca agt ggg ccg agc gac tct gag aca gac 1440
Pro Thr Val Ser Gln Glu Pro Ser Gly Pro Ser Asp Ser Glu Thr Asp
465 470 475 480

tgc atg tat ggg aat ggc aaa gac tat cgg ggc aaa acg gcc gtc act 1488
Cys Met Tyr Gly Asn Gly Lys Asp Tyr Arg Gly Lys Thr Ala Val Thr
485 490 495

gca gct ggc acc ccc tgc cag gga tgg gct gcc cag gag ccc cac agg 1536
Ala Ala Gly Thr Pro Cys Gln Gly Trp Ala Ala Gln Glu Pro His Arg
500 505 510

cac agc atc ttc acc cca cag aca aac cca cgg gca gat ctg gaa aag 1584
His Ser Ile Phe Thr Pro Gln Thr Asn Pro Arg Ala Asp Leu Glu Lys
515 520 525

aac tac tgc cga aac cca gat ggg gat gtg aat ggt cct tgg tgc tat 1632
Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Asn Gly Pro Trp Cys Tyr
530 535 540

aca aca aac ccc aga aaa ctt tat gac tat tgt gac atc ccc ctg tgt 1680
Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Ile Pro Leu Cys
545 550 555 560

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gca tca gca tca tcc ttt gag tgc ggg aaa cct cag gtg gaa ccg aag      1728
Ala Ser Ala Ser Ser Phe Glu Cys Gly Lys Pro Gln Val Glu Pro Lys
                565                 570                 575

aaa tgc cct ggg agg gtg gtg ggt ggc tgc gtg gcc aac cct cac tcc      1776
Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala Asn Pro His Ser
            580                 585                 590

tgg ccc tgg caa atc agc ctt aga aca aga ttt acc gga cag cac ttc      1824
Trp Pro Trp Gln Ile Ser Leu Arg Thr Arg Phe Thr Gly Gln His Phe
        595                 600                 605

tgt ggc ggt act tta ata gcc cca gag tgg gtt ctg act gct gcc cac      1872
Cys Gly Gly Thr Leu Ile Ala Pro Glu Trp Val Leu Thr Ala Ala His
    610                 615                 620

tgt ttg gag aaa tct tca aga cct gaa ttc tac aag gtt atc ctg ggt      1920
Cys Leu Glu Lys Ser Ser Arg Pro Glu Phe Tyr Lys Val Ile Leu Gly
625                 630                 635                 640

gcg cac gaa gaa tat atc cgt ggg ttg gat gtt cag gaa ata tca gta      1968
Ala His Glu Glu Tyr Ile Arg Gly Leu Asp Val Gln Glu Ile Ser Val
                645                 650                 655

gcc aaa ctg atc ttg gag ccc aac aac cgt gac att gcc ctg ctg aaa      2016
Ala Lys Leu Ile Leu Glu Pro Asn Asn Arg Asp Ile Ala Leu Leu Lys
            660                 665                 670

cta agc cgc cca gcc acc atc acg gat aaa gtc att cca gct tgt ctg      2064
Leu Ser Arg Pro Ala Thr Ile Thr Asp Lys Val Ile Pro Ala Cys Leu
        675                 680                 685

cca tct cca aat tac atg gtt gct gac cgg aca ata tgt tac atc acc      2112
Pro Ser Pro Asn Tyr Met Val Ala Asp Arg Thr Ile Cys Tyr Ile Thr
    690                 695                 700

ggc tgg gga gag act caa ggg act ttc ggt gcc ggt cgt ctc aag gag      2160
Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Arg Leu Lys Glu
705                 710                 715                 720

gct cag ctg cct gtg att gag aac aag gtg tgc aac cgc gtc gag tat      2208
Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Val Glu Tyr
                725                 730                 735

ctg aac aac aga gtc aaa tcc acg gag ctc tgt gcc ggg caa ctg gct      2256
Leu Asn Asn Arg Val Lys Ser Thr Glu Leu Cys Ala Gly Gln Leu Ala
            740                 745                 750

ggt ggc gtc gac agc tgc caa ggc gac agt gga gga cct ctg gtt tgc      2304
Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
        755                 760                 765

ttc gag aag gac aag tac att tta caa gga gtc act tct tgg ggt ctt      2352
Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu
    770                 775                 780
```

ggc tgt gct cgc ccc aat aag cct ggt gtc tac gtt cgt gtc tca cgg 2400
Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg
785 790 795 800

ttt gtt gat tgg att gaa agg gag atg agg aat aac tgactaggtg 2446
Phe Val Asp Trp Ile Glu Arg Glu Met Arg Asn Asn
805 810

gaaggccgag caaaacctct gcttactaaa gcttactgaa tatggggaga cggcttaggg 2506
tgtttggaaa aactgacagt aatcaaactg ggacactaca ctgaaccaca gcttcctgtc 2566
gcccctcagc ccctcccctt tttttgtatt attgtgggta aaattttcct gtctgtggac 2626
ttctggattt tgtgacaata gaccatcact gctgtgacct ttgttgaaaa taaactcgat 2686
acttactttg 2696

<210> 4
<211> 812
<212> PRT
<213> Mus musculus

<400> 4
Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Leu Lys Pro
1 5 10 15
Gly Gln Gly Asp Ser Leu Asp Gly Tyr Ile Ser Thr Gln Gly Ala Ser
20 25 30
Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp
35 40 45
Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe
50 55 60
Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser
65 70 75 80
Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys
85 90 95
Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg
100 105 110
Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly
115 120 125
Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn
130 135 140
Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln
145 150 155 160
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys
165 170 175
Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys
180 185 190
Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala
195 200 205
Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe
210 215 220
Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu
225 230 235 240
Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr
245 250 255
Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr
260 265 270

```
Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser
        275                 280                 285
Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro
    290                 295                 300
His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu
305                 310                 315                 320
Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
                325                 330                 335
Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys
            340                 345                 350
Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu
        355                 360                 365
Glu Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser
    370                 375                 380
Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser
385                 390                 395                 400
Trp Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe
                405                 410                 415
Pro Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp
            420                 425                 430
Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445
Cys Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly Ser Val Val Glu Leu
    450                 455                 460
Pro Thr Val Ser Gln Glu Pro Ser Gly Pro Ser Asp Ser Glu Thr Asp
465                 470                 475                 480
Cys Met Tyr Gly Asn Gly Lys Asp Tyr Arg Gly Lys Thr Ala Val Thr
                485                 490                 495
Ala Ala Gly Thr Pro Cys Gln Gly Trp Ala Ala Gln Glu Pro His Arg
            500                 505                 510
His Ser Ile Phe Thr Pro Gln Thr Asn Pro Arg Ala Asp Leu Glu Lys
        515                 520                 525
Asn Tyr Cys Arg Asn Pro Asp Gly Asp Val Asn Gly Pro Trp Cys Tyr
    530                 535                 540
Thr Thr Asn Pro Arg Lys Leu Tyr Asp Tyr Cys Asp Ile Pro Leu Cys
545                 550                 555                 560
Ala Ser Ala Ser Ser Phe Glu Cys Gly Lys Pro Gln Val Glu Pro Lys
                565                 570                 575
Lys Cys Pro Gly Arg Val Val Gly Gly Cys Val Ala Asn Pro His Ser
            580                 585                 590
Trp Pro Trp Gln Ile Ser Leu Arg Thr Arg Phe Thr Gly Gln His Phe
        595                 600                 605
Cys Gly Gly Thr Leu Ile Ala Pro Glu Trp Val Leu Thr Ala Ala His
    610                 615                 620
Cys Leu Glu Lys Ser Ser Arg Pro Glu Phe Tyr Lys Val Ile Leu Gly
625                 630                 635                 640
Ala His Glu Glu Tyr Ile Arg Gly Leu Asp Val Gln Glu Ile Ser Val
                645                 650                 655
Ala Lys Leu Ile Leu Glu Pro Asn Asn Arg Asp Ile Ala Leu Leu Lys
            660                 665                 670
Leu Ser Arg Pro Ala Thr Ile Thr Asp Lys Val Ile Pro Ala Cys Leu
        675                 680                 685
Pro Ser Pro Asn Tyr Met Val Ala Asp Arg Thr Ile Cys Tyr Ile Thr
    690                 695                 700
Gly Trp Gly Glu Thr Gln Gly Thr Phe Gly Ala Gly Arg Leu Lys Glu
```

705 710 715 720
Ala Gln Leu Pro Val Ile Glu Asn Lys Val Cys Asn Arg Val Glu Tyr
725 730 735
Leu Asn Asn Arg Val Lys Ser Thr Glu Leu Cys Ala Gly Gln Leu Ala
740 745 750
Gly Gly Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Val Cys
755 760 765
Phe Glu Lys Asp Lys Tyr Ile Leu Gln Gly Val Thr Ser Trp Gly Leu
770 775 780
Gly Cys Ala Arg Pro Asn Lys Pro Gly Val Tyr Val Arg Val Ser Arg
785 790 795 800
Phe Val Asp Trp Ile Glu Arg Glu Met Arg Asn Asn
805 810

<210> 5
<211> 1083
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)...(1083)

<400> 5
tat ctc tca gag tgc aag act ggg aat gga aag aac tac aga ggg acg 48
Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg Gly Thr
1 5 10 15

atg tcc aaa aca aaa aat ggc atc acc tgt caa aaa tgg agt tcc act 96
Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser Ser Thr
20 25 30

tct ccc cac aga cct aga ttc tca cct gct aca cac ccc tca gag gga 144
Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser Glu Gly
35 40 45

ctg gag gag aac tac tgc agg aat cca gac aac gat ccg cag ggg ccc 192
Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln Gly Pro
50 55 60

tgg tgc tat act act gat cca gaa aag aga tat gac tac tgc gac att 240
Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys Asp Ile
65 70 75 80

ctt gag tgt gaa gag gaa tgt atg cat tgc agt gga gaa aac tat gac 288
Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn Tyr Asp
85 90 95

ggc aaa att tcc aag acc atg tct gga ctg gaa tgc cag gcc tgg gac 336
Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala Trp Asp
100 105 110

tct cag agc cca cac gct cat gga tac att cct tcc aaa ttt cca aac 384
Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe Pro Asn
115 120 125

```
aag aac ctg aag aag aat tac tgt cgt aac ccc gat agg gag ctg cgg      432
Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Arg Glu Leu Arg
    130                 135                 140

cct tgg tgt ttc acc acc gac ccc aac aag cgc tgg gaa ctt tgc gac      480
Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu Cys Asp
145                 150                 155                 160

atc ccc cgc tgc aca aca cct cca cca tct tct ggt ccc acc tac cag      528
Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr Tyr Gln
                165                 170                 175

tgt ctg aag gga aca ggt gaa aac tat cgc ggg aat gtg gct gtt acc      576
Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala Val Thr
            180                 185                 190

gtt tcc ggg cac acc tgt cag cac tgg agt gca cag acc cct cac aca      624
Val Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr Pro His Thr
        195                 200                 205

cat aac agg aca cca gaa aac ttc ccc tgc aaa aat ttg gat gaa aac      672
His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp Glu Asn
    210                 215                 220

tac tgc cgc aat cct gac gga aaa agg gcc cca tgg tgc cat aca acc      720
Tyr Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys His Thr Thr
225                 230                 235                 240

aac agc caa gtg cgg tgg gag tac tgt aag ata ccg tcc tgt gac tcc      768
Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys Asp Ser
                245                 250                 255

tcc cca gta tcc acg gaa caa ttg gct ccc aca gca cca cct gag cta      816
Ser Pro Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro Glu Leu
            260                 265                 270

acc cct gtg gtc cag gac tgc tac cat ggt gat gga cag agc tac cga      864
Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser Tyr Arg
        275                 280                 285

ggc aca tcc tcc acc acc acc aca gga aag aag tgt cag tct tgg tca      912
Gly Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser Trp Ser
    290                 295                 300

tct atg aca cca cac cgg cac cag aag acc cca gaa aac tac cca aat      960
Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr Pro Asn
305                 310                 315                 320

gct ggc ctg aca atg aac tac tgc agg aat cca gat gcc gat aaa ggc     1008
Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp Lys Gly
                325                 330                 335

ccc tgg tgt ttt acc aca gac ccc agc gtc agg tgg gag tac tgc aac     1056
Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr Cys Asn
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340 345 350

ctg aaa aaa tgc tca gga aca gaa gcg 1083
Leu Lys Lys Cys Ser Gly Thr Glu Ala
355 360

<210> 6
<211> 361
<212> PRT
<213> Homo sapiens

<400> 6
Tyr Leu Ser Glu Cys Lys Thr Gly Asn Gly Lys Asn Tyr Arg Gly Thr
1 5 10 15
Met Ser Lys Thr Lys Asn Gly Ile Thr Cys Gln Lys Trp Ser Ser Thr
20 25 30
Ser Pro His Arg Pro Arg Phe Ser Pro Ala Thr His Pro Ser Glu Gly
35 40 45
Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Pro Gln Gly Pro
50 55 60
Trp Cys Tyr Thr Thr Asp Pro Glu Lys Arg Tyr Asp Tyr Cys Asp Ile
65 70 75 80
Leu Glu Cys Glu Glu Glu Cys Met His Cys Ser Gly Glu Asn Tyr Asp
85 90 95
Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Glu Cys Gln Ala Trp Asp
100 105 110
Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ser Lys Phe Pro Asn
115 120 125
Lys Asn Leu Lys Lys Asn Tyr Cys Arg Asn Pro Asp Arg Glu Leu Arg
130 135 140
Pro Trp Cys Phe Thr Thr Asp Pro Asn Lys Arg Trp Glu Leu Cys Asp
145 150 155 160
Ile Pro Arg Cys Thr Thr Pro Pro Pro Ser Ser Gly Pro Thr Tyr Gln
165 170 175
Cys Leu Lys Gly Thr Gly Glu Asn Tyr Arg Gly Asn Val Ala Val Thr
180 185 190
Val Ser Gly His Thr Cys Gln His Trp Ser Ala Gln Thr Pro His Thr
195 200 205
His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Asp Glu Asn
210 215 220
Tyr Cys Arg Asn Pro Asp Gly Lys Arg Ala Pro Trp Cys His Thr Thr
225 230 235 240
Asn Ser Gln Val Arg Trp Glu Tyr Cys Lys Ile Pro Ser Cys Asp Ser
245 250 255
Ser Pro Val Ser Thr Glu Gln Leu Ala Pro Thr Ala Pro Pro Glu Leu
260 265 270
Thr Pro Val Val Gln Asp Cys Tyr His Gly Asp Gly Gln Ser Tyr Arg
275 280 285
Gly Thr Ser Ser Thr Thr Thr Thr Gly Lys Lys Cys Gln Ser Trp Ser
290 295 300
Ser Met Thr Pro His Arg His Gln Lys Thr Pro Glu Asn Tyr Pro Asn
305 310 315 320
Ala Gly Leu Thr Met Asn Tyr Cys Arg Asn Pro Asp Ala Asp Lys Gly
325 330 335

```
Pro Trp Cys Phe Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr Cys Asn
            340                 345                 350
Leu Lys Lys Cys Ser Gly Thr Glu Ala
        355                 360

      <210> 7
      <211> 1086
      <212> DNA
      <213> Mus musculus

      <220>
      <221> CDS
      <222> (1)...(1086)

      <400> 7
gtg tat ctg tca gaa tgt aag acc ggc atc ggc aac ggc tac aga gga       48
Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg Gly
 1               5                  10                  15

acc atg tcc agg aca aag agt ggt gtt gcc tgt caa aag tgg ggt gcc       96
Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly Ala
            20                  25                  30

acg ttc ccc cac gta ccc aac tac tct ccc agt aca cat ccc aat gag      144
Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn Glu
        35                  40                  45

gga cta gaa gag aac tac tgt agg aac cca gac aat gat gaa caa ggg      192
Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln Gly
    50                  55                  60

cct tgg tgc tac act aca gat ccg gac aag aga tat gac tac tgc aac      240
Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys Asn
65                  70                  75                  80

att cct gaa tgt gaa gag gaa tgc atg tac tgc agt gga gaa aag tat      288
Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys Tyr
                85                  90                  95

gag ggc aaa atc tcc aag acc atg tct gga ctt gac tgc cag gcc tgg      336
Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala Trp
            100                 105                 110

gat tct cag agc cca cat gct cat gga tac atc cct gcc aaa ttt cca      384
Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe Pro
        115                 120                 125

agc aag aac ctg aag atg aat tat tgc cac aac cct gac ggg gag cca      432
Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu Pro
    130                 135                 140

agg ccc tgg tgc ttc aca aca gac ccc acc aaa cgc tgg gaa tac tgt      480
Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr Cys
145                 150                 155                 160
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```
gac atc ccc cgc tgc aca aca ccc ccg ccc cca ccc agc cca acc tac        528
Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr Tyr
                165                     170                     175

caa tgt ctg aaa gga aga ggt gaa aat tac cga ggg acc gtg tct gtc        576
Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser Val
            180                     185                     190

acc gtg tct ggg aaa acc tgt cag cgc tgg agt gag caa acc cct cat        624
Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro His
        195                     200                     205

agg cac aac agg aca cca gaa aat ttc ccc tgc aaa aat ctg gaa gag        672
Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu Glu
    210                     215                     220

aac tac tgc cgg aac cca gat gga gaa act gct ccc tgg tgc tat acc        720
Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr Thr
225                     230                     235                     240

act gac agc cag ctg agg tgg gag tac tgt gag att cca tcc tgc gag        768
Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys Glu
                245                     250                     255

tcc tca gca tca cca gac cag tca gat tcc tca gtt cca cca gag gag        816
Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu Glu
            260                     265                     270

caa aca cct gtg gtc cag gaa tgc tac cag agc gat ggg cag agc tat        864
Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser Tyr
        275                     280                     285

cgg ggt aca tcg tcc act acc atc aca ggg aag aag tgc cag tcc tgg        912
Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser Trp
    290                     295                     300

gca gct atg ttt cca cac agg cat tcg aag acc cca gag aac ttc cca        960
Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe Pro
305                     310                     315                     320

gat gct ggc ttg gag atg aac tac tgc agg aac ccg gat ggt gac aag       1008
Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp Lys
                325                     330                     335

ggc cct tgg tgc tac acc act gac ccg agc gtc agg tgg gaa tac tgc       1056
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr Cys
            340                     345                     350

aac ctg aag cgg tgc tca gag aca gga ggg                               1086
Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly
        355                     360
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<210> 8
<211> 362

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<212> PRT
<213> Mus musculus

<400> 8
Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg Gly
 1               5                  10                  15
Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly Ala
            20                  25                  30
Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn Glu
        35                  40                  45
Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln Gly
    50                  55                  60
Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys Asn
65                  70                  75                  80
Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys Tyr
                85                  90                  95
Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala Trp
            100                 105                 110
Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe Pro
        115                 120                 125
Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu Pro
    130                 135                 140
Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr Cys
145                 150                 155                 160
Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr Tyr
                165                 170                 175
Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser Val
            180                 185                 190
Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro His
        195                 200                 205
Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu Glu
    210                 215                 220
Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr Thr
225                 230                 235                 240
Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys Glu
                245                 250                 255
Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu Glu
            260                 265                 270
Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser Tyr
        275                 280                 285
Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser Trp
    290                 295                 300
Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe Pro
305                 310                 315                 320
Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp Lys
                325                 330                 335
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr Cys
            340                 345                 350
Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly
        355                 360

<210> 9
<211> 552
<212> DNA
<213> Mus musculus
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<220>
<221> CDS
<222> (1)...(552)

<400> 9

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cat act cat cag gac ttt cag cca gtg ctc cac ctg gtg gca ctg aac        48
His Thr His Gln Asp Phe Gln Pro Val Leu His Leu Val Ala Leu Asn
 1               5                  10                  15

acc ccc ctg tct gga ggc atg cgt ggt atc cgt gga gca gat ttc cag        96
Thr Pro Leu Ser Gly Gly Met Arg Gly Ile Arg Gly Ala Asp Phe Gln
            20                  25                  30

tgc ttc cag caa gcc cga gcc gtg ggg ctg tcg ggc acc ttc cgg gct       144
Cys Phe Gln Gln Ala Arg Ala Val Gly Leu Ser Gly Thr Phe Arg Ala
        35                  40                  45

ttc ctg tcc tct agg ctg cag gat ctc tat agc atc gtg cgc cgt gct       192
Phe Leu Ser Ser Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg Arg Ala
    50                  55                  60

gac cgg ggg tct gtg ccc atc gtc aac ctg aag gac gag gtg cta tct       240
Asp Arg Gly Ser Val Pro Ile Val Asn Leu Lys Asp Glu Val Leu Ser
65                  70                  75                  80

ccc agc tgg gac tcc ctg ttt tct ggc tcc cag ggt caa gtg caa ccc       288
Pro Ser Trp Asp Ser Leu Phe Ser Gly Ser Gln Gly Gln Val Gln Pro
                85                  90                  95

ggg gcc cgc atc ttt tct ttt gac ggc aga gat gtc ctg aga cac cca       336
Gly Ala Arg Ile Phe Ser Phe Asp Gly Arg Asp Val Leu Arg His Pro
            100                 105                 110

gcc tgg ccg cag aag agc gta tgg cac ggc tcg gac ccc agt ggg cgg       384
Ala Trp Pro Gln Lys Ser Val Trp His Gly Ser Asp Pro Ser Gly Arg
        115                 120                 125

agg ctg atg gag agt tac tgt gag aca tgg cga act gaa act act ggg       432
Arg Leu Met Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr Thr Gly
    130                 135                 140

gct aca ggt cag gcc tcc tcc ctg ctg tca ggc agg ctc ctg gaa cag       480
Ala Thr Gly Gln Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu Glu Gln
145                 150                 155                 160

aaa gct gcg agc tgc cac aac agc tac atc gtc ctg tgc att gag aat       528
Lys Ala Ala Ser Cys His Asn Ser Tyr Ile Val Leu Cys Ile Glu Asn
                165                 170                 175

agc ttc atg acc tct ttc tcc aaa                                       552
Ser Phe Met Thr Ser Phe Ser Lys
            180
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<210> 10

<211> 184
<212> PRT
<213> Mus musculus

<400> 10
His Thr His Gln Asp Phe Gln Pro Val Leu His Leu Val Ala Leu Asn
1 5 10 15
Thr Pro Leu Ser Gly Gly Met Arg Gly Ile Arg Gly Ala Asp Phe Gln
20 25 30
Cys Phe Gln Gln Ala Arg Ala Val Gly Leu Ser Gly Thr Phe Arg Ala
35 40 45
Phe Leu Ser Ser Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg Arg Ala
50 55 60
Asp Arg Gly Ser Val Pro Ile Val Asn Leu Lys Asp Glu Val Leu Ser
65 70 75 80
Pro Ser Trp Asp Ser Leu Phe Ser Gly Ser Gln Gly Gln Val Gln Pro
85 90 95
Gly Ala Arg Ile Phe Ser Phe Asp Gly Arg Asp Val Leu Arg His Pro
100 105 110
Ala Trp Pro Gln Lys Ser Val Trp His Gly Ser Asp Pro Ser Gly Arg
115 120 125
Arg Leu Met Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr Thr Gly
130 135 140
Ala Thr Gly Gln Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu Glu Gln
145 150 155 160
Lys Ala Ala Ser Cys His Asn Ser Tyr Ile Val Leu Cys Ile Glu Asn
165 170 175
Ser Phe Met Thr Ser Phe Ser Lys
180

<210> 11
<211> 1414
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(1414)

<400> 11
atg gac cat aag gaa gta atc ctt ctg ttt ctc ttg ctt ctg aaa cca 48
Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Leu Lys Pro
1 5 10 15

gga caa ggg gac tcg ctg gat ggc tac ata agc aca caa ggg gct tca 96
Gly Gln Gly Asp Ser Leu Asp Gly Tyr Ile Ser Thr Gln Gly Ala Ser
20 25 30

ctg ttc agt ctc acc aag aag cag ctc gca gca gga ggt gtc tcg gac 144
Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp
35 40 45

tgt ttg gcc aaa tgt gaa ggg gaa aca gac ttt gtc tgc agg tca ttc 192
Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe
50 55 60

cag tac cac agc aaa gag cag caa tgc gtg atc atg gcg gag aac agc 240
Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser
65 70 75 80

aag act tcc tcc atc atc cgg atg aga gac gtc atc tta ttc gaa aag 288
Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys
85 90 95

aga gtg tat ctg tca gaa tgt aag acc ggc atc ggc aac ggc tac aga 336
Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg
100 105 110

gga acc atg tcc agg aca aag agt ggt gtt gcc tgt caa aag tgg ggt 384
Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly
115 120 125

gcc acg ttc ccc cac gta ccc aac tac tct ccc agt aca cat ccc aat 432
Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn
130 135 140

gag gga cta gaa gag aac tac tgt agg aac cca gac aat gat gaa caa 480
Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln
145 150 155 160

ggg cct tgg tgc tac act aca gat ccg gac aag aga tat gac tac tgc 528
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys
165 170 175

aac att cct gaa tgt gaa gag gaa tgc atg tac tgc agt gga gaa aag 576
Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys
180 185 190

tat gag ggc aaa atc tcc aag acc atg tct gga ctt gac tgc cag gcc 624
Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala
195 200 205

tgg gat tct cag agc cca cat gct cat gga tac atc cct gcc aaa ttt 672
Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe
210 215 220

cca agc aag aac ctg aag atg aat tat tgc cac aac cct gac ggg gag 720
Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu
225 230 235 240

cca agg ccc tgg tgc ttc aca aca gac ccc acc aaa cgc tgg gaa tac 768
Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr
245 250 255

tgt gac atc ccc cgc tgc aca aca ccc ccg ccc cca ccc agc cca acc 816
Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr
260 265 270

tac caa tgt ctg aaa gga aga ggt gaa aat tac cga ggg acc gtg tct 864
Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser

```
            275                 280                 285

gtc acc gtg tct ggg aaa acc tgt cag cgc tgg agt gag caa acc cct      912
Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro
    290                 295                 300

cat agg cac aac agg aca cca gaa aat ttc ccc tgc aaa aat ctg gaa      960
His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu
305                 310                 315                 320

gag aac tac tgc cgg aac cca gat gga gaa act gct ccc tgg tgc tat     1008
Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
                325                 330                 335

acc act gac agc cag ctg agg tgg gag tac tgt gag att cca tcc tgc     1056
Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys
            340                 345                 350

gag tcc tca gca tca cca gac cag tca gat tcc tca gtt cca cca gag     1104
Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu
        355                 360                 365

gag caa aca cct gtg gtc cag gaa tgc tac cag agc gat ggg cag agc     1152
Glu Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser
    370                 375                 380

tat cgg ggt aca tcg tcc act acc atc aca ggg aag aag tgc cag tcc     1200
Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser
385                 390                 395                 400

tgg gca gct atg ttt cca cac agg cat tcg aag acc cca gag aac ttc     1248
Trp Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe
                405                 410                 415

cca gat gct ggc ttg gag atg aac tac tgc agg aac ccg gat ggt gac     1296
Pro Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp
            420                 425                 430

aag ggc cct tgg tgc tac acc act gac ccg agc gtc agg tgg gaa tac     1344
Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445

tgc aac ctg aag cgg tgc tca gag aca gga ggg aat tca gac tac aag     1392
Cys Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly Asn Ser Asp Tyr Lys
    450                 455                 460

gac gac gat gac aag taa taa c                                       1414
Asp Asp Asp Asp Lys  *   *
465
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<210> 12
<211> 469
<212> PRT
<213> Mus musculus

<400> 12

Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Leu Lys Pro
1 5 10 15
Gly Gln Gly Asp Ser Leu Asp Gly Tyr Ile Ser Thr Gln Gly Ala Ser
20 25 30
Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp
35 40 45
Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe
50 55 60
Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser
65 70 75 80
Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys
85 90 95
Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg
100 105 110
Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly
115 120 125
Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn
130 135 140
Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln
145 150 155 160
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys
165 170 175
Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys
180 185 190
Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala
195 200 205
Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe
210 215 220
Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu
225 230 235 240
Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr
245 250 255
Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr
260 265 270
Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser
275 280 285
Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro
290 295 300
His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu
305 310 315 320
Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
325 330 335
Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys
340 345 350
Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu
355 360 365
Glu Gln Thr Pro Val Val Gln Glu Cys Tyr Gln Ser Asp Gly Gln Ser
370 375 380
Tyr Arg Gly Thr Ser Ser Thr Thr Ile Thr Gly Lys Lys Cys Gln Ser
385 390 395 400
Trp Ala Ala Met Phe Pro His Arg His Ser Lys Thr Pro Glu Asn Phe
405 410 415
Pro Asp Ala Gly Leu Glu Met Asn Tyr Cys Arg Asn Pro Asp Gly Asp
420 425 430

```
Lys Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ser Val Arg Trp Glu Tyr
        435                 440                 445
Cys Asn Leu Lys Arg Cys Ser Glu Thr Gly Gly Asn Ser Asp Tyr Lys
    450                 455                 460
Asp Asp Asp Asp Lys
465

      <210> 13
      <211> 661
      <212> DNA
      <213> Mus musculus

      <220>
      <221> CDS
      <222> (1)...(661)

      <400> 13
atg gac cat aag gaa gta atc ctt ctg ttt ctc ttg ctt ctg aaa cca        48
Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Leu Lys Pro
 1               5                  10                  15

gga caa ggg gac tcg cta gat ctt gac tac aag gac gac gat gac aag        96
Gly Gln Gly Asp Ser Leu Asp Leu Asp Tyr Lys Asp Asp Asp Asp Lys
            20                  25                  30

ctt gct cat act cat cag gac ttt cag cca gtg ctc cac ctg gtg gca       144
Leu Ala His Thr His Gln Asp Phe Gln Pro Val Leu His Leu Val Ala
        35                  40                  45

ctg aac acc ccc ctg tct gga ggc atg cgt ggt atc cgt gga gca gat       192
Leu Asn Thr Pro Leu Ser Gly Gly Met Arg Gly Ile Arg Gly Ala Asp
    50                  55                  60

ttc cag tgc ttc cag caa gcc cga gcc gtg ggg ctg tcg ggc acc ttc       240
Phe Gln Cys Phe Gln Gln Ala Arg Ala Val Gly Leu Ser Gly Thr Phe
 65                  70                  75                  80

cgg gct ttc ctg tcc tct agg ctg cag gat ctc tat agc atc gtg cgc       288
Arg Ala Phe Leu Ser Ser Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg
                 85                  90                  95

cgt gct gac cgg ggg tct gtg ccc atc gtc aac ctg aag gac gag gtg       336
Arg Ala Asp Arg Gly Ser Val Pro Ile Val Asn Leu Lys Asp Glu Val
            100                 105                 110

cta tct ccc agc tgg gac tcc ctg ttt tct ggc tcc cag ggt caa gtg       384
Leu Ser Pro Ser Trp Asp Ser Leu Phe Ser Gly Ser Gln Gly Gln Val
        115                 120                 125

caa ccc ggg gcc cgc atc ttt tct ttt gac ggc aga gat gtc ctg aga       432
Gln Pro Gly Ala Arg Ile Phe Ser Phe Asp Gly Arg Asp Val Leu Arg
    130                 135                 140

cac cca gcc tgg ccg cag aag agc gta tgg cac ggc tcg gac ccc agt       480
His Pro Ala Trp Pro Gln Lys Ser Val Trp His Gly Ser Asp Pro Ser
```

```
145                 150                 155                 160

ggg cgg agg ctg atg gag agt tac tgt gag aca tgg cga act gaa act      528
Gly Arg Arg Leu Met Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr
                165                 170                 175

act ggg gct aca ggt cag gcc tcc tcc ctg ctg tca ggc agg ctc ctg      576
Thr Gly Ala Thr Gly Gln Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu
            180                 185                 190

gaa cag aaa gct gcg agc tgc cac aac agc tac atc gtc ctg tgc att      624
Glu Gln Lys Ala Ala Ser Cys His Asn Ser Tyr Ile Val Leu Cys Ile
        195                 200                 205

gag aat agc ttc atg acc tct ttc tcc aaa taa taa c                    661
Glu Asn Ser Phe Met Thr Ser Phe Ser Lys  *   *
    210                 215
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<210> 14
<211> 218
<212> PRT
<213> Mus musculus

<400> 14

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Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Leu Lys Pro
 1               5                  10                  15
Gly Gln Gly Asp Ser Leu Asp Leu Asp Tyr Lys Asp Asp Asp Asp Lys
            20                  25                  30
Leu Ala His Thr His Gln Asp Phe Gln Pro Val Leu His Leu Val Ala
        35                  40                  45
Leu Asn Thr Pro Leu Ser Gly Gly Met Arg Gly Ile Arg Gly Ala Asp
    50                  55                  60
Phe Gln Cys Phe Gln Gln Ala Arg Ala Val Gly Leu Ser Gly Thr Phe
65                  70                  75                  80
Arg Ala Phe Leu Ser Ser Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg
                85                  90                  95
Arg Ala Asp Arg Gly Ser Val Pro Ile Val Asn Leu Lys Asp Glu Val
            100                 105                 110
Leu Ser Pro Ser Trp Asp Ser Leu Phe Ser Gly Ser Gln Gly Gln Val
        115                 120                 125
Gln Pro Gly Ala Arg Ile Phe Ser Phe Asp Gly Arg Asp Val Leu Arg
    130                 135                 140
His Pro Ala Trp Pro Gln Lys Ser Val Trp His Gly Ser Asp Pro Ser
145                 150                 155                 160
Gly Arg Arg Leu Met Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr
                165                 170                 175
Thr Gly Ala Thr Gly Gln Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu
            180                 185                 190
Glu Gln Lys Ala Ala Ser Cys His Asn Ser Tyr Ile Val Leu Cys Ile
        195                 200                 205
Glu Asn Ser Phe Met Thr Ser Phe Ser Lys
    210                 215
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<210> 15

<211> 1747
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(1747)

<400> 15

atg gac cat aag gaa gta atc ctt ctg ttt ctc ttg ctt ctg aaa cca 48
Met Asp His Lys Glu Val Ile Leu Leu Phe Leu Leu Leu Leu Lys Pro
1 5 10 15

gga caa ggg gac tcg ctg gat ggc tac ata agc aca caa ggg gct tca 96
Gly Gln Gly Asp Ser Leu Asp Gly Tyr Ile Ser Thr Gln Gly Ala Ser
20 25 30

ctg ttc agt ctc acc aag aag cag ctc gca gca gga ggt gtc tcg gac 144
Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp
35 40 45

tgt ttg gcc aaa tgt gaa ggg gaa aca gac ttt gtc tgc agg tca ttc 192
Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe
50 55 60

cag tac cac agc aaa gag cag caa tgc gtg atc atg gcg gag aac agc 240
Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser
65 70 75 80

aag act tcc tcc atc atc cgg atg aga gac gtc atc tta ttc gaa aag 288
Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys
85 90 95

aga gtg tat ctg tca gaa tgt aag acc ggc atc ggc aac ggc tac aga 336
Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg
100 105 110

gga acc atg tcc agg aca aag agt ggt gtt gcc tgt caa aag tgg ggt 384
Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly
115 120 125

gcc acg ttc ccc cac gta ccc aac tac tct ccc agt aca cat ccc aat 432
Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn
130 135 140

gag gga cta gaa gag aac tac tgt agg aac cca gac aat gat gaa caa 480
Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln
145 150 155 160

ggg cct tgg tgc tac act aca gat ccg gac aag aga tat gac tac tgc 528
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys
165 170 175

aac att cct gaa tgt gaa gag gaa tgc atg tac tgc agt gga gaa aag 576
Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys

```
            180                       185                       190

tat gag ggc aaa atc tcc aag acc atg tct gga ctt gac tgc cag gcc        624
Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala
        195                     200                     205

tgg gat tct cag agc cca cat gct cat gga tac atc cct gcc aaa ttt        672
Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe
    210                     215                     220

cca agc aag aac ctg aag atg aat tat tgc cac aac cct gac ggg gag        720
Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu
225                     230                     235                     240

cca agg ccc tgg tgc ttc aca aca gac ccc acc aaa cgc tgg gaa tac        768
Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr
                245                     250                     255

tgt gac atc ccc cgc tgc aca aca ccc ccg ccc cca ccc agc cca acc        816
Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr
            260                     265                     270

tac caa tgt ctg aaa gga aga ggt gaa aat tac cga ggg acc gtg tct        864
Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser
        275                     280                     285

gtc acc gtg tct ggg aaa acc tgt cag cgc tgg agt gag caa acc cct        912
Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro
    290                     295                     300

cat agg cac aac agg aca cca gaa aat ttc ccc tgc aaa aat ctg gaa        960
His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu
305                     310                     315                     320

gag aac tac tgc cgg aac cca gat gga gaa act gct ccc tgg tgc tat       1008
Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
                325                     330                     335

acc act gac agc cag ctg agg tgg gag tac tgt gag att cca tcc tgc       1056
Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys
            340                     345                     350

gag tcc tca gca tca cca gac cag tca gat tcc tca gtt cca cca gag       1104
Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu
        355                     360                     365

gag caa aca cct gtg gga ggg aat tgc ggc ggt gga tca ggt ggc gga       1152
Glu Gln Thr Pro Val Gly Gly Asn Cys Gly Gly Gly Ser Gly Gly Gly
    370                     375                     380

gat ctt gac tac aag gac gac gat gac aag ctt gct cat act cat cag       1200
Asp Leu Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala His Thr His Gln
385                     390                     395                     400

gac ttt cag cca gtg ctc cac ctg gtg gca ctg aac acc ccc ctg tct       1248
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Asp Phe Gln Pro Val Leu His Leu Val Ala Leu Asn Thr Pro Leu Ser
405 410 415

gga ggc atg cgt ggt atc cgt gga gca gat ttc cag tgc ttc cag caa 1296
Gly Gly Met Arg Gly Ile Arg Gly Ala Asp Phe Gln Cys Phe Gln Gln
420 425 430

gcc cga gcc gtg ggg ctg tcg ggc acc ttc cgg gct ttc ctg tcc tct 1344
Ala Arg Ala Val Gly Leu Ser Gly Thr Phe Arg Ala Phe Leu Ser Ser
435 440 445

agg ctg cag gat ctc tat agc atc gtg cgc cgt gct gac cgg ggg tct 1392
Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg Arg Ala Asp Arg Gly Ser
450 455 460

gtg ccc atc gtc aac ctg aag gac gag gtg cta tct ccc agc tgg gac 1440
Val Pro Ile Val Asn Leu Lys Asp Glu Val Leu Ser Pro Ser Trp Asp
465 470 475 480

tcc ctg ttt tct ggc tcc cag ggt caa gtg caa ccc ggg gcc cgc atc 1488
Ser Leu Phe Ser Gly Ser Gln Gly Gln Val Gln Pro Gly Ala Arg Ile
485 490 495

ttt tct ttt gac ggc aga gat gtc ctg aga cac cca gcc tgg ccg cag 1536
Phe Ser Phe Asp Gly Arg Asp Val Leu Arg His Pro Ala Trp Pro Gln
500 505 510

aag agc gta tgg cac ggc tcg gac ccc agt ggg cgg agg ctg atg gag 1584
Lys Ser Val Trp His Gly Ser Asp Pro Ser Gly Arg Arg Leu Met Glu
515 520 525

agt tac tgt gag aca tgg cga act gaa act act ggg gct aca ggt cag 1632
Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr Thr Gly Ala Thr Gly Gln
530 535 540

gcc tcc tcc ctg ctg tca ggc agg ctc ctg gaa cag aaa gct gcg agc 1680
Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu Glu Gln Lys Ala Ala Ser
545 550 555 560

tgc cac aac agc tac atc gtc ctg tgc att gag aat agc ttc atg acc 1728
Cys His Asn Ser Tyr Ile Val Leu Cys Ile Glu Asn Ser Phe Met Thr
565 570 575

tct ttc tcc aaa taa taa c 1747
Ser Phe Ser Lys * *
580

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<211> 580
<212> PRT
<213> Mus musculus

<400> 16
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Leu Phe Ser Leu Thr Lys Lys Gln Leu Ala Ala Gly Gly Val Ser Asp
35 40 45
Cys Leu Ala Lys Cys Glu Gly Glu Thr Asp Phe Val Cys Arg Ser Phe
50 55 60
Gln Tyr His Ser Lys Glu Gln Gln Cys Val Ile Met Ala Glu Asn Ser
65 70 75 80
Lys Thr Ser Ser Ile Ile Arg Met Arg Asp Val Ile Leu Phe Glu Lys
85 90 95
Arg Val Tyr Leu Ser Glu Cys Lys Thr Gly Ile Gly Asn Gly Tyr Arg
100 105 110
Gly Thr Met Ser Arg Thr Lys Ser Gly Val Ala Cys Gln Lys Trp Gly
115 120 125
Ala Thr Phe Pro His Val Pro Asn Tyr Ser Pro Ser Thr His Pro Asn
130 135 140
Glu Gly Leu Glu Glu Asn Tyr Cys Arg Asn Pro Asp Asn Asp Glu Gln
145 150 155 160
Gly Pro Trp Cys Tyr Thr Thr Asp Pro Asp Lys Arg Tyr Asp Tyr Cys
165 170 175
Asn Ile Pro Glu Cys Glu Glu Glu Cys Met Tyr Cys Ser Gly Glu Lys
180 185 190
Tyr Glu Gly Lys Ile Ser Lys Thr Met Ser Gly Leu Asp Cys Gln Ala
195 200 205
Trp Asp Ser Gln Ser Pro His Ala His Gly Tyr Ile Pro Ala Lys Phe
210 215 220
Pro Ser Lys Asn Leu Lys Met Asn Tyr Cys His Asn Pro Asp Gly Glu
225 230 235 240
Pro Arg Pro Trp Cys Phe Thr Thr Asp Pro Thr Lys Arg Trp Glu Tyr
245 250 255
Cys Asp Ile Pro Arg Cys Thr Thr Pro Pro Pro Pro Pro Ser Pro Thr
260 265 270
Tyr Gln Cys Leu Lys Gly Arg Gly Glu Asn Tyr Arg Gly Thr Val Ser
275 280 285
Val Thr Val Ser Gly Lys Thr Cys Gln Arg Trp Ser Glu Gln Thr Pro
290 295 300
His Arg His Asn Arg Thr Pro Glu Asn Phe Pro Cys Lys Asn Leu Glu
305 310 315 320
Glu Asn Tyr Cys Arg Asn Pro Asp Gly Glu Thr Ala Pro Trp Cys Tyr
325 330 335
Thr Thr Asp Ser Gln Leu Arg Trp Glu Tyr Cys Glu Ile Pro Ser Cys
340 345 350
Glu Ser Ser Ala Ser Pro Asp Gln Ser Asp Ser Ser Val Pro Pro Glu
355 360 365
Glu Gln Thr Pro Val Gly Gly Asn Cys Gly Gly Gly Ser Gly Gly Gly
370 375 380
Asp Leu Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala His Thr His Gln
385 390 395 400
Asp Phe Gln Pro Val Leu His Leu Val Ala Leu Asn Thr Pro Leu Ser
405 410 415
Gly Gly Met Arg Gly Ile Arg Gly Ala Asp Phe Gln Cys Phe Gln Gln
420 425 430
Ala Arg Ala Val Gly Leu Ser Gly Thr Phe Arg Ala Phe Leu Ser Ser
435 440 445

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Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg Arg Ala Asp Arg Gly Ser
    450                 455                 460
Val Pro Ile Val Asn Leu Lys Asp Glu Val Leu Ser Pro Ser Trp Asp
465                 470                 475                 480
Ser Leu Phe Ser Gly Ser Gln Gly Gln Val Gln Pro Gly Ala Arg Ile
                485                 490                 495
Phe Ser Phe Asp Gly Arg Asp Val Leu Arg His Pro Ala Trp Pro Gln
            500                 505                 510
Lys Ser Val Trp His Gly Ser Asp Pro Ser Gly Arg Arg Leu Met Glu
        515                 520                 525
Ser Tyr Cys Glu Thr Trp Arg Thr Glu Thr Thr Gly Ala Thr Gly Gln
    530                 535                 540
Ala Ser Ser Leu Leu Ser Gly Arg Leu Leu Glu Gln Lys Ala Ala Ser
545                 550                 555                 560
Cys His Asn Ser Tyr Ile Val Leu Cys Ile Glu Asn Ser Phe Met Thr
                565                 570                 575
Ser Phe Ser Lys
            580
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<210> 17
<211> 549
<212> DNA
<213> Homo sapiens

<400> 17

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gggctggcgg gcaccttccg cgccttcctg tcctcgcgcc tgcaggacct gtacagcatc     180
gtgcgccgtg ccgaccgcgc agccgtgccc atcgtcaacc tcaaggacga gctgctgttt     240
cccagctggg aggctctgtt ctcaggctct gagggtccgc tgaagcccgg ggcacgcatc     300
ttctcctttg acggcaagga cgtcctgagg caccccacct ggccccagaa gagcgtgtgg     360
catggctcgg accccaacgg gcgcaggctg accgagagct actgtgagac gtggcggacg     420
gaggctccct cggccacggg ccaggcctcc tcgctgctgg ggggcaggct cctggggcag     480
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<210> 18
<211> 183
<212> PRT
<213> Homo sapiens

<400> 18

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Ser Pro Leu Ser Gly Gly Met Arg Gly Ile Arg Gly Ala Asp Phe Gln
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Cys Phe Gln Gln Ala Arg Ala Val Gly Leu Ala Gly Thr Phe Arg Ala
        35                  40                  45
Phe Leu Ser Ser Arg Leu Gln Asp Leu Tyr Ser Ile Val Arg Arg Ala
    50                  55                  60
Asp Arg Ala Ala Val Pro Ile Val Asn Leu Lys Asp Glu Leu Leu Phe
65                  70                  75                  80
Pro Ser Trp Glu Ala Leu Phe Ser Gly Ser Glu Gly Pro Leu Lys Pro
                85                  90                  95
Gly Ala Arg Ile Phe Ser Phe Asp Gly Lys Asp Val Leu Arg His Pro
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100 105 110

Thr Trp Pro Gln Lys Ser Val Trp His Gly Ser Asp Pro Asn Gly Arg

115 120 125

Arg Leu Thr Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Ala Pro Ser

130 135 140

Ala Thr Gly Gln Ala Ser Ser Leu Leu Gly Gly Arg Leu Leu Gly Gln

145 150 155 160

Ser Ala Ala Ser Cys His His Ala Tyr Ile Val Leu Cys Ile Glu Asn

165 170 175

Ser Phe Met Thr Ala Ser Lys

180

<210> 19
<211> 5408
<212> DNA
<213> Homo sapiens

<400> 19
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ccctggtcct gctgctcggg gtccgcgcgg cctccgcgga gccagagcgc atcagcgagg 180
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aaaaaaaa 5408

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<211> 1336
<212> PRT
<213> Homo sapiens

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20 25 30
Ala Glu Pro Glu Arg Ile Ser Glu Glu Val Gly Leu Leu Gln Leu Leu
35 40 45
Gly Asp Pro Pro Pro Gln Gln Val Thr Gln Thr Asp Asp Pro Asp Val
50 55 60
Gly Leu Ala Tyr Val Phe Gly Pro Asp Ala Asn Ser Gly Gln Val Ala
65 70 75 80
Arg Tyr His Phe Pro Ser Leu Phe Phe Arg Asp Phe Ser Leu Leu Phe
85 90 95
His Ile Arg Pro Ala Thr Glu Gly Pro Gly Val Leu Phe Ala Ile Thr
100 105 110
Asp Ser Ala Gln Ala Met Val Leu Leu Gly Val Lys Leu Ser Gly Val
115 120 125
Gln Asp Gly His Gln Asp Ile Ser Leu Leu Tyr Thr Glu Pro Gly Ala
130 135 140
Gly Gln Thr His Thr Ala Ala Ser Phe Arg Leu Pro Ala Phe Val Gly
145 150 155 160
Gln Trp Thr His Leu Ala Leu Ser Val Ala Gly Gly Phe Val Ala Leu
165 170 175
Tyr Val Asp Cys Glu Glu Phe Gln Arg Met Pro Leu Ala Arg Ser Ser
180 185 190
Arg Gly Leu Glu Leu Glu Pro Gly Ala Gly Leu Phe Val Ala Gln Ala
195 200 205
Gly Gly Ala Asp Pro Asp Lys Phe Gln Gly Val Ile Ala Glu Leu Lys
210 215 220
Val Arg Arg Asp Pro Gln Val Ser Pro Met His Cys Leu Asp Glu Glu
225 230 235 240
Gly Asp Asp Ser Asp Gly Ala Phe Gly Asp Ser Gly Ser Gly Leu Gly
245 250 255
Asp Ala Arg Glu Leu Leu Arg Glu Glu Thr Gly Ala Ala Leu Lys Pro
260 265 270
Arg Leu Pro Ala Pro Pro Pro Val Thr Thr Pro Pro Leu Ala Gly Gly
275 280 285
Ser Ser Thr Glu Asp Ser Arg Ser Glu Glu Val Glu Glu Gln Thr Thr
290 295 300
Val Ala Ser Leu Gly Ala Gln Thr Leu Pro Gly Ser Asp Ser Val Ser
305 310 315 320
Thr Trp Asp Gly Ser Val Arg Thr Pro Gly Gly Arg Val Lys Glu Gly
325 330 335
Gly Leu Lys Gly Gln Lys Gly Glu Pro Gly Val Pro Gly Pro Pro Gly
340 345 350
Arg Ala Gly Pro Pro Gly Ser Pro Cys Leu Pro Gly Pro Pro Gly Leu
355 360 365
Pro Cys Pro Val Ser Pro Leu Gly Pro Ala Gly Pro Ala Leu Gln Thr
370 375 380

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Val Pro Gly Pro Gln Gly Pro Pro Gly Pro Pro Gly Arg Asp Gly Thr
385                 390                 395                 400
Pro Gly Arg Asp Gly Glu Pro Gly Asp Pro Gly Glu Asp Gly Lys Pro
                405                 410                 415
Gly Asp Thr Gly Pro Gln Gly Phe Pro Gly Thr Pro Gly Asp Val Gly
            420                 425                 430
Pro Lys Gly Asp Lys Gly Asp Pro Gly Val Gly Glu Arg Gly Pro Pro
        435                 440                 445
Gly Pro Gln Gly Pro Pro Gly Pro Pro Gly Pro Ser Phe Arg His Asp
    450                 455                 460
Lys Leu Thr Phe Ile Asp Met Glu Gly Ser Gly Phe Gly Gly Asp Leu
465                 470                 475                 480
Glu Ala Leu Arg Gly Pro Arg Gly Phe Pro Gly Pro Pro Gly Pro Pro
                485                 490                 495
Gly Val Pro Gly Leu Pro Gly Glu Pro Gly Arg Phe Gly Val Asn Ser
            500                 505                 510
Ser Asp Val Pro Gly Pro Ala Gly Leu Pro Gly Val Pro Gly Arg Glu
        515                 520                 525
Gly Pro Pro Gly Phe Pro Gly Leu Pro Gly Pro Pro Gly Pro Pro Gly
    530                 535                 540
Arg Glu Gly Pro Pro Gly Arg Thr Gly Gln Lys Gly Ser Leu Gly Glu
545                 550                 555                 560
Ala Gly Ala Pro Gly His Lys Gly Ser Lys Gly Ala Pro Gly Pro Ala
                565                 570                 575
Gly Ala Arg Gly Glu Ser Gly Leu Ala Gly Ala Pro Gly Pro Ala Gly
            580                 585                 590
Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Gly Leu Pro
        595                 600                 605
Ala Gly Phe Asp Asp Met Glu Gly Ser Gly Gly Pro Phe Trp Ser Thr
    610                 615                 620
Ala Arg Ser Ala Asp Gly Pro Gln Gly Pro Pro Gly Leu Pro Gly Leu
625                 630                 635                 640
Lys Gly Asp Pro Gly Val Pro Gly Leu Pro Gly Ala Lys Gly Glu Val
                645                 650                 655
Gly Ala Asp Gly Ile Pro Gly Phe Pro Gly Leu Pro Gly Arg Glu Gly
            660                 665                 670
Ile Ala Gly Pro Gln Gly Pro Lys Gly Asp Arg Gly Ser Arg Gly Glu
        675                 680                 685
Lys Gly Asp Pro Gly Lys Asp Gly Val Gly Gln Pro Gly Leu Pro Gly
    690                 695                 700
Pro Pro Gly Pro Pro Gly Pro Val Val Tyr Val Ser Glu Gln Asp Gly
705                 710                 715                 720
Ser Val Leu Ser Val Pro Gly Pro Glu Gly Arg Pro Gly Phe Ala Gly
                725                 730                 735
Phe Pro Gly Pro Ala Gly Pro Lys Gly Asn Leu Gly Ser Lys Gly Glu
            740                 745                 750
Arg Gly Ser Pro Gly Pro Lys Gly Glu Lys Gly Glu Pro Gly Ser Ile
        755                 760                 765
Phe Ser Pro Asp Gly Gly Ala Leu Gly Pro Ala Gln Lys Gly Ala Lys
    770                 775                 780
Gly Glu Pro Gly Phe Arg Gly Pro Pro Gly Pro Tyr Gly Arg Pro Gly
785                 790                 795                 800
Tyr Lys Gly Glu Ile Gly Phe Pro Gly Arg Pro Gly Arg Pro Gly Met
                805                 810                 815
Asn Gly Leu Lys Gly Glu Lys Gly Glu Pro Gly Asp Ala Ser Leu Gly
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Gly Pro Pro Gly Thr Pro Val Tyr Asp Ser Asn Val Phe Ala Glu Ser
850 855 860
Ser Arg Pro Gly Pro Pro Gly Leu Pro Gly Asn Gln Gly Pro Pro Gly
865 870 875 880
Pro Lys Gly Ala Lys Gly Glu Val Gly Pro Pro Gly Pro Pro Gly Gln
885 890 895
Phe Pro Phe Asp Phe Leu Gln Leu Glu Ala Glu Met Lys Gly Glu Lys
900 905 910
Gly Asp Arg Gly Asp Ala Gly Gln Lys Gly Glu Arg Gly Glu Pro Gly
915 920 925
Gly Gly Gly Phe Phe Gly Ser Ser Leu Pro Gly Pro Pro Gly Pro Pro
930 935 940
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945 950 955 960
Arg Gly Gln Pro Gly Pro Pro Gly Pro Gln Gly Pro Pro Gly Ile Gly
965 970 975
Tyr Glu Gly Arg Gln Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro
980 985 990
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995 1000 1005
Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Thr Met Gly Ala Ser
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1075 1080 1085
Pro Pro Val Val Gln Leu His Asp Ser Asn Pro Tyr Pro Arg Arg Glu
1090 1095 1100
His Pro His Pro Thr Ala Arg Pro Trp Arg Ala Asp Asp Ile Leu Ala
1105 1110 1115 112
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1125 1130 1135
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1170 1175 1180
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1205 1210 1215
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1235 1240 1245
Pro Gly Ala Arg Ile Phe Ser Phe Asp Gly Lys Asp Val Leu Arg His
1250 1255 1260

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1265                1270                1275                128
Arg Arg Leu Thr Glu Ser Tyr Cys Glu Thr Trp Arg Thr Glu Ala Pro
                1285                1290                1295
Ser Ala Thr Gly Gln Ala Ser Ser Leu Leu Gly Gly Arg Leu Leu Gly
            1300                1305                1310
Gln Ser Ala Ala Ser Cys His His Ala Tyr Ile Val Leu Cys Ile Glu
        1315                1320                1325
Asn Ser Phe Met Thr Ala Ser Lys
    1330                1335
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/24950

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :A01N 63/00, 43/04; C12N 15/00; C07H 21/02
US CL : 424/93.1; 435/320.1; 514/44; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.1; 435/320.1; 514/44; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y,P | US 5,792,845 A (O'REILLY et al.) 11 August 1998 (11.08.98), col. 4, lines 32-68, col. 5, lines 1-2, 51-68, col. 6, lines 1-8. | 1-30, 33 |
| Y | WO 97/23500 A1 (THE CHILDREN'S MEDICAL CENTER CORPORATION) 03 July 1997 (03.07.97), page 41, lines 3-33, page 42, lines 1-27. | 4 |
| X,P<br>------<br>Y,P | WO 98/49321 A2 (RHONE-POULENC RORER) 05 November 1998 (05.11.98), page 44, 6-11, 25-33, page 45, lines 12-13, 29-35. | 1, 5, 18, 20, 31<br>------<br>2-4, 6-17, 19, 21-30, 32, 33 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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| | |
|---|---|
| Date of the actual completion of the international search<br>04 FEBRUARY 1999 | Date of mailing of the international search report<br>08 MAR 1999 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231 | Authorized officer<br>SHIN-LIN CHEN |
| Facsimile No. (703) 305-3230 | Telephone No. (703) 308-0196 |

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 97/15666 A (THE CHILDREN'S MEDICAL CENTER CORPORATION) 01 May 1997 (01.05.97), page 20, lines 16-35, page 21, page 22, lines 1-16. page 59, lines 5-35, page 60, page 61, 1-6. | 1-30, 33 |
| Y | TANAKA, T. et al. Retroviral and adenoviral mediated transduction of angiostatin cDNA inhibits angiogenesis and tumor growth. Proceedings of the American Association for Cancer Research. March 1997 (03.97). Vol 38. page 264. | 1-33 |
| Y | WO 96/35774 A2 (THE CHILDREN'S MEDICAL CENTER CORPORATION) 14 November 1996 (14.11.96), page lines 33-36, pages 22-25, page 26, lines 1-33. pages 144-148. | 1-30, 33 |
| Y | WO 97/41824 A2(ABBOTT LABORATORIES) 13 November 1997 (13.11.97), page 5, lines 13-38, page 6, 1-18, page 60, lines 15-38, pages 61-62, page 63, lines 1-33. | 1-30, 33 |
| Y | WO 95/29242 A1 (THE CHILDREN;S MEDICAL CENTER CORPORATION) 02 November 1995 (02.11.95), page 21, lines 19-35, pages 22-27, page 28, lines 1-4. page 87, lines 4-35, page 88, page 89, lines 1-14. | 1-30, 33 |
| Y | O'REILLY, et al. Angiostatin induces and sustains dormancy of human primary tumors in mice. Nature Medicine, June 1996 (06.96), Vol. 2, No. 6, pages 689-692, especially pages 689-690. | 1-30, 33 |
| Y | O'REILLY, et al. Endostatin: An Endogenous Inhibitor of Angiogenesis and Tumor Growth. Cell, 24 January 1997 (24.01.97), Vol. 88, pages 277-285, especially pages 279-280, 282. | 1-30, 33 |

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN, WPIDS, MEDLINE, CAPLUS, BIOSIS, EMBASE
search terms: angiostatin, plasminogen, endostatin, colagen(w) XVIII, inhibit?(5a)tumor(5a)growth, tumor(5a)regress?, diabet?(p)retinopathy, plasmid, viral(5a) vector.